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Applicant(s): ERNEST P. NOBLE, ROSS YOUNG, and BRUCE LAWFORD

Docket: G&C 30435.154-US-P1

Title: GENETIC MARKER OF RESPONSE TO ATYPICAL ANTIPSYCHOTICS AND ANTIDEPRESSANTS AND METHODS FOR USE THEREOF

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(1) Full Name Of Inventor	Family Name NOBLE	First Given Name ERNEST	Second Given Name P.
Residence & Citizenship	City Laguna Beach	State or Foreign Country California	Country of Citizenship USA
Post Office Address	Post Office Address	City Laguna Beach	State & Zip Code/Country California USA
(2) Full Name Of Inventor	Family Name YOUNG	First Given Name ROSS	Second Given Name
Residence & Citizenship	City Brisbane	State or Foreign Country Australia	Country of Citizenship New Zealand
Post Office Address	Post Office Address	City	State & Zip Code/Country Australia
(3) Full Name Of Inventor	Family Name LAWFORD	First Given Name BRUCE	Second Given Name
Residence & Citizenship	City Brisbane	State or Foreign Country Australia	Country of Citizenship Australia
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GATES & COOPER LLP
Howard Hughes Center
6701 Center Drive West, Suite 1050
Los Angeles, CA 90045
(310) 641-8797

By: _____

Name: Karen S. Canady
Reg. No.: 39,927
Initials: KSC/sjm

GENETIC MARKER OF RESPONSE TO ATYPICAL ANTIPSYCHOTICS AND ANTIDEPRESSANTS AND METHODS FOR USE THEREOF

Throughout this application various publications are referenced. The disclosures of these

5   publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

The treatment of patients with antipsychotic medications is hindered by side-effects and complications that cause considerable distress and result in low adherence to medications.

10   Older, "typical" antipsychotics bind tightly to the D2 dopamine receptor (DRD2) and some patients experience poor response to treatment with these medications. Currently used medications, known as atypical antipsychotics, bind to the D2dopamine receptor with varying strength. While the atypical medications represent a significant advance in the treatment of psychosis, there are no guidelines to match patients to medication type to

15   both maximize clinical response and to minimize the likelihood of harmful adverse effects, such as extrapyramidal movement symptoms and diabetes.

Similarly, response to the class of antidepressant medications known as selective serotonin reuptake inhibitors (SSRI) has been variable. SSRIs are an effective treatment for a wide variety of psychiatric disorders for many patients, but some patients do not respond to

20   medication. There remains a need for predictors of patient response to assist clinicians in the selection of antidepressant as well as antipsychotic medications.

SUMMARY OF THE INVENTION

The invention provides methods of identifying candidate psychiatric patientsor patients with movement disorder for treatment with medication that acts at the D2 dopamine

25   receptor. The method comprises determining a patient's D2 dopamine receptor (DRD2) genotype. Patients having the Taq1A (A1) allele (A1+ allelic status) are candidates for treatment with high dose of high D2 dopamine receptor binding antipsychotics and/or SSRIs that influence D2 dopamine receptor density. Patients lacking the Taq1A allele (A1-

1

allelic status) are candidates for treatment with lowdose of low D2 dopamine receptor binding or low dose high D2 dopamine receptor binding atypical antipsychotics, and are not likely to respond well tothese SSRIs.

5   In one embodiment, the psychiatric patient suffers from schizophrenia. In another embodiment, the patient suffers from PTSD, depression, social anxiety or mixed anxiety and depressive states. In a third embodiment, the patient suffers from a movement disorder, such as Parkinson's Disease. It is accepted psychiatric practice to treat a schizophrenic or other psychotic patient with either a high binding antipsychotic, examples of which include Risperidone, Flupenthixol, Fluphenazine decanoate, Zuclopenthixol,

10 Haloperidol, Thiondazine, Thiothixene or Trofluperazine, or a low binding atypical antipsychotics, such as Olanzapine, Clozapine. Patients suffering from PTSD, depression, social anxiety or mixed anxiety and depressive states are typically treated with an SSRI, such as Paroxetine or Halopram.

EXAMPLES

15 The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

Example 1: Dose related extrapyramidal adverse effects in schizophrenic patients treated with Risperidone are associated with the D2 dopamine receptor gene.

20 **Introduction**

The effective treatment of schizophrenia with antipsychotic medication is frequently compromised by adverse effects. Medication induced Extrapyramidal effects (EPS) are associated with decreased compliance and poorer treatment outcome (Gerlach, 2002).

25 Additionally EPS are associated with worsened negative symptoms, dysphoria, impaired cognition and an increased risk of tardive dyskinesia (Tandon, 2002). Anticholinergic medication can be efficacious in ameliorating EPS however these compounds are associated with their own adverse effects such as sexual dysfunction, constipation, dry mouth, urinary retention, impaired cognition and an exacerbation of psychosis (Kopala,

30 1997). It remains an important clinical challenge to identify medications and dose regimes with strong clinical efficacy and tolerable adverse effect profiles.

Risperidone, a second generation atypical antipsychotic medication, is associated with a lower occurrence of EPS (Love & Nelson, 2000; Heck et al 2000) and tardive

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dyskinesia (Jeste, et. al. 1999) when compared with conventional, typical antipsychotics. While this has been an important treatment advance EPS are more frequently associated with this medication as dose increases (Sussman, 2002, Love & Nelson, 2000) as a result of increased D2dopamine receptor occupancy (Nyberg et al, 1999).

5   Consequently, the recommended dose of Risperidone has been reduced from 6mg/day to 4mg/day, although some patients still experience EPS at this lower dose (Nyberg et al 1999).

10   The dose of Risperidone that induces EPS varies considerably among patients suggesting differing individual susceptibility. (Nyberg et al, 1999) Individual, physiological factors that influence the development of EPS have not been identified. One such factor may be the Taq1 A restriction fragment length polymorphism located in the untranslated region of theD2 dopamine receptor DRD2 gene (Grevle et al, 2000).

15   The A1 (variant) allele (A1/A1 and A1/A2 genotypes) is more commonly found in patients with Parkinson's Disease than in matched controls (Grevle et al 2000) and is also associated with motor fluctuation in affected individuals (Wang et al 2002). Furthermore, this polymorphism is associated with a raised prolactin response to antipsychotic medication (Mihara, 2000, 2001; Young et al in press) reflecting greater

20   antipsychotic D2 occupancy (Markianos et al 2001). As the Taq 1A polymorphism of the DRD2 is associated with both Parkinson's disease and increased antipsychotic D2dopamine occupancy we investigated whether or not patients treated with Risperidone showed differing dose susceptibility for EPS.

25   **Method.**

    SUBJECTS. Forty-seven Caucasian patients attending three psychiatric units for the treatment of their schizophrenia consented for the study. All patients met Diagnostic and Statistical Manual of Mental Disorders (DSM-1V) criteria for schizophrenia. They

30   underwent a clinical history taking by either a psychiatrist (SB, BL, MB, WW) or a clinical psychologist (R.McDY). All participants also underwent a neurological examination and demographic details including ethnic background data was obtained. All subjects had received Risperidone for at least one month at a stable dose. The current dose varied between 2mg and 6 mg Risperidone per day, 2mg (N=9), 2.5 mg

35   (N=1), 3mg (N=13), 4mg (N=16), 4.5 mg (N=1), 5 mg (N=2), and 6mg (N=5). There were no significant differences in dose between A1+ and A1- subjects. Adherence in inpatients was sound as all inpatients were administered medication by nursing staff. Outpatient adherence was estimated by self-report and assessment by their treating psychiatrists.

40

    As other psychoactive agents can influence dopaminergic activity (Jarvinen et al., 1992, Hugues et al 2000, Keltner et al 2002, Basturk et al 2001) those patients on regular antidepressant, opiate, anxiolytic or mood stabilising medication were excluded from the study. All patients were physically well. The average age of those recruited was

45   33.8 years (sd=12.2 years), and there were 41 males and 8 females. Of the sample, 18

were inpatients at the time of data collection and 31 were outpatients. In terms of clinical and psychosocial history 12 reported a past history of suicide attempts and 13 had a past history of criminal conviction.

5   PROCEDURE   Patients were recruited from the Fortitude Valley Community Mental Health Centre, the Royal Brisbane Mental Health Unit and the Park Psychiatric Hospital. These facilities are located in Brisbane, Australia. Inclusion criteria included being aged between 18 and 65 years , having a DSM IV diagnosis of Schizophrenia and also being cognitively able to participate in order to maximise the validity of data

10   obtained. All subjects possessed an adequate comprehension of English. Potential participants were excluded if they had Schizoaffective Disorder or any other major comorbid psychiatric disorder such as Bipolar Disorder, Dementia, Organic Brain Syndrome, and Major Depressive Disorder with Delusions . EPS were assessed using the scales "Parkinsonism, Dystonia and Dyskinesia: Questionnaire and Behavioral

15   scale(Physician Or Nurse) and "Parkinsonism: Physician's Examination" from the Extrapyramidal Rating Scale (ESRS), (Chounaird, et al , 1980) the most comprehensive rating scale for EPS (De Deyn & Wirshing, 2001). The Questionnaire and Behavioral scale involves a Physician inquiring about, and then rating, symptoms of Parkinsonism, dystonia and dyskinesia. The Physician's examination involves rating expressive

20   automatic movements, bradykinesia, rigidity, gait and posture, tremor, akathisia, sialorrhea, and postural stability.

A 10mL blood sample was drawn from each subject for DNA extraction and prolactin determination. DNA was sent to the U.C.L.A. for genotyping. All participants provided

25   informed consent and were able to terminate participation at any time without prejudice. Institutional ethics approval was obtained from the clinics and hospitals involved. Demographic and clinical data were combined with genetic data at the completion of the trial.

Genotyping

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GENOTYPING

A ten ml. blood sample was drawn from each subject. DNA was extracted using standard techniques and subsequently used as a template for determination of Taq1A DRD2 alleles by polymerase chain reaction (Grandy et al 1993). As previously

35   described (Noble et al 1994) the amplification of DNA was carried out using a Perkin Elmer Gene Amp 9600 thermocycler. Approximately 500ng of amplified DNA was then digested with 5 units of Taq 1 restriction enzyme (GIBCO/BRL, Grand Island, NY) at 65 degrees centigrade overnight. The resulting products were analysed by electrophoresis in a 2.5% agarose gel containing ethidium bromide and visualised under

40   ultraviolet light. The A1/A2 genotype is revealed by three fragments: 310bp, 180bp, and 130bp. A2/A2 genotype is indicated by two fragments: 180bp and 130bp. The A1/A1 genotype is shown by the uncleaved 310bp fragment.

Data Analysis

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Information coded from interview proformas was entered into a computer data base. Doses of Risperidone were converted to chlorpromazine equivalents per kilo of body weight. A median split was performed in order to separate individuals receiving median dose or above (high dose,n=24) from those receiving below median dose(low dose
5   ,n=23) Subjects were then divided into four groups ; A1 subjects receiving below the median dose risperidone, (A1 low dose); A1 subjects receiving the median dose or above of risperidone, (A1 high dose), A2 subjects treated with below the median dose,(A2 low dose); A2 subjects treated with the median dose or above, (A2 high dose). Univariate analysis of variance (ANOVA) was used to test between- subject effects. A
10 p-value of <0.05 was considered to be statistically significant.

Results

The 47 subjects had the following genotypes: A1A1(A1 allele) n=3, A1A2 (A1 allele), n
15 =23 ; and A2A2 (A2 allele), $n = 21$.   There was no significant difference in the ages of the high and low dose groups (df1,45; F=2.14,p=0.150) and A1+ and A1- allelic subjects . Figure(1) presents mean ESRS Parkinsonism Physician's Examination scores of the four groups ;A1 low dose (n=15); A1high dose, (n=11); A2 low dose, (n=8); A2 high dose, (n=13) The means and standard deviations of these ESRS scores
20 in particular groups were :A1 low dose, mean=4.53, S.D.=4.357;   A1 high dose ,mean=2.18, S.D.=2.56; A2 low dose, mean=2.63, S.D.=3.29; A2 high dose, mean=8.54, S.D.=6.00. There was a significant gene by dose interaction (df 1,43, F=5.189, p=0.028). At high doses of Risperidone A2 subjects were rated by physicians as having significantly worse symptoms of Parkinsonism than A1 subjects.(df 1,23,
25 F=4.790, p=0.039) At low doses there was a trend for A1 subjects having more severe symptoms of Parkinsonism than A2 subjects.(df 1,21, F=3.4, p=0.08)

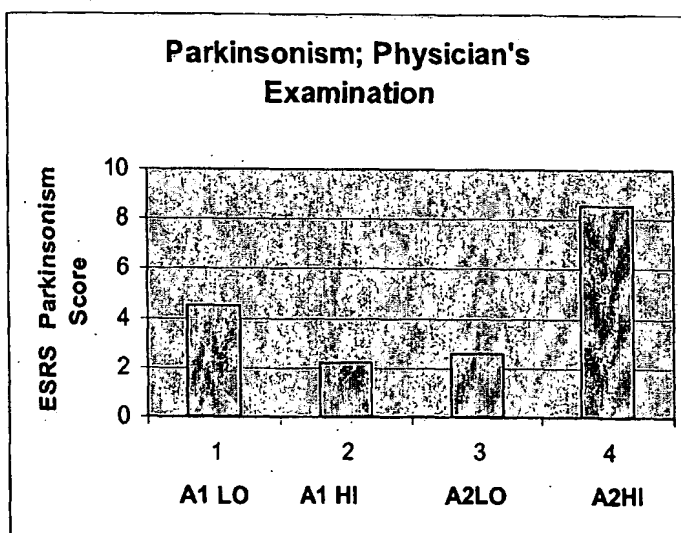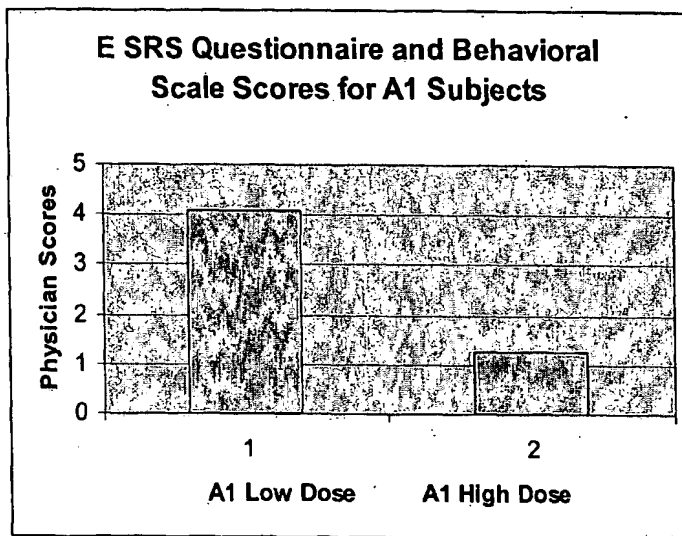Parkinsonism; Physician's Examination

Figure 1 ESRS Parkinsonism ; Physician's Examination Scores of subjects in the four different allele/dosage groups. There was a significant allele by dose interaction (p=0.028)

Figure (2) shows ESRS Parkinsonism, Dystonia and Dyskinesia: Questionnaire and
5   Behavioral scale(Physician Or Nurse) scores for A1 subjects treated with high and low dose Risperidone. .Low dose A1 subjects displayed significantly worse symptoms (mean score =4.07,s.d.=2.80) than A1 subjects given high doses of Risperidone(mean score = 1.27,s.d.=1.62).(df=1,24, F=8.8, p=0.0067)

E SRS Questionnaire and Behavioral Scale Scores for A1 Subjects

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Figure(2) A1 patients receiving below median doses of Risperidone(A1 Low Dose) had significantly worse symptoms of Parkinsonism, Dystonia and Dyskinesia than A1 patients treated with median dose or above. (p=0.0067)

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Discussion

The essential finding of this study is that A1 and A2 individuals exhibit different dose
20   susceptibility for EPS when treated with Risperidone. This may be due to the marked differences in D2 physiology between these groups.

There is compelling evidence that A1+ individuals have a reduced density of brain D2 dopamine receptors (Noble et al,1991 ,Thompson et al, 1997, Pohjalainen et al, 1998, Jonsson, et al, 1999). An early brain autopsy study (Noble et al., 1991) found a
25   significant reduction of approximately 30% in the number of D2 dopamine receptors (Bmax) in the caudate nucleus of A1+ compared to A1- allelic subjects. Moreover, a significant progressive decline in Bmax was found across A2/A2, A1/A2, A1/A1 genotypes in that order. There was no difference in D2 dopamine binding affinity (Kd) between A1+ and A1- allelic subjects. Thompson et al., 1997 also reported 30%-40%
30   reduction in D2 dopamine receptor density in the striatum of A1+ individuals compared to A1- individuals. An in-vivo study of healthy Finnish volunteers (Pohjalainen et al.,

1998) showed significantly decreased D2 receptor density in the striatum of A1+ when compared with A1- allelic subjects. Again, there was no difference in the Kd between the two groups. In Jonsson et al (1999), another PET study of healthy humans using (11C) raclopride, a significant association of the A1 allele with low D2 dopamine
5   receptor density was found. While, Laruelle et al (1998) reported no association between reduced D2 binding and the A1 allele subjects an editorial (Hitzemann, 1998) suggested the study had insufficient power to detect a significant difference between A1+ and A1- individuals.

10   The most likely explanation of the association of theA1 allele, a polymorphism in a non-coding region of the DRD2 gene, with reduced D2 receptor density, is that the sequence variation causing the Taq1 A polymorphism is in linkage disequilibrium with functional allelic variants that affect receptor expression (Noble, et al, 1998, Comings et al, 1991, O'Hara et al 1993, Arinami et al, 1997). The Taq 1A variants are now known to be in
15   linkage disequilibrium with C957T, a synonymous mutation in the human DRD2. Furthermore, C957T affects mRNA folding leading to both less stable mRNA and decreased translation. These effects dramatically diminish dopamine induced up-regulation of D2 receptors (Duan et al, 2003).
     A1+ individuals with genetically determined reduced D2 density have D2 receptors that
20   are functionally identical to those found in A1- individuals. The binding affinity for dopamine (Kd) is identical in both A1+ and A1- individuals.(Noble et al 1991, Pohjalainen 1998) A1+ individuals will, therefore, at any given dose of antipsychotic medication, tend to have a lower density of free, unbound, D2 receptors .and consequently, greater drug D2 occupancy. Additionally A1 individuals have a
25   diminished ability to upregulate D2 receptors in response to blockade (Duan et al ,2003). A1 individuals have significantly worse Parkinsonian symptoms at doses below the median than at doses above the median. Furthermore A2 subjects treated with above median doses of Risperidone are rated as having worse EPSES than A1 subjects treated with the same dose of medication. The susceptibility of A1 subjects at low doses of
30   Risperidone probably reflects the lower density of free, unbound D2 receptors found in this group. Higher doses of Risperidone are required to induce EPSES in A2 individuals due to their greater D2 density and greater capacity for D2 upregulation. At high doses of Risperidone, A1 subjects experience minimal EPSES. Why this occurs is not known however high doses (>100mg.per day) of haloperidol employed in the 1970s for
35   treatment resistant schizophrenia were not associated with serious extrapyramidal side effects. (McCreadie Rg and MacDonald IM, 1977) Lower density of D2 receptors found in A1 subjects combined with a higher dose of the "tight binding" agent Risperidone (David et al 2000) is likely to result in very few unbound D2 receptors in this group of patients. Perhaps very high D2 drug occupancy results in a suppression of
40   EPSES.

     This study is an association study of a clinical population. A prospective study of the EPSES associated with Risperidone dosage encountered in A1 and A2 patients is necessary in order to fully explore the observations presented in this paper.

If confirmed, these observations are likely to be of significant practical assistance to treating clinicians in allowing prediction of patients who are likely to experience Parkinsonian symptoms at low antipsychotic dose and will provide a rational strategy for consequent drug dosing. For example, this may indicate that in A1- patients with
5   EPS the dose should not be increased but in A1+ patients an increased dose may ameliorate the symptoms. This may also result in an improved antipsychotic effect in A1+ patients.

10   References

Arinami T, Gao M, Hamaguchi H, Toru M,(1997) A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. Human Molecular Genetics 6:577.
15

Chouinard G, Ross-Chouinard A, Annable L, Jones B (1980) The extrapyramidal symptoms rating scale. Can J Neurol Sci 7:233

Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, Knell E,
20   Kocsis P, Baumgarten R, Kovacs BW, Levy DL, Smith M, Borison RL, Evans DD, Klein DN, MacMurray J, Tosk JF, Sverd J, Gysin R, Flanagan SD,. (1991) The dopamine D2 locus as a modifying gene in neuropsychiatric disorders. Journal of the American Medical Association. 266:1793-1800

25   David SR , Taylor CC, Kinon BJ, Breier A. (2000) The effects of olanzapine, risperidone, and haloperidol on plasma prolactin levels in patients with schizophrenia. Clin Ther; 9:1085-96.

De Duyn PP, Wirshung WC. (2001) Scales to assess efficacy and safety of
30   pharmacologic agents in the treatment of behavioral and psychological symptoms of dementia. J Clin Psychiatry;62 Suppl 21:19-22

Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV (2003) Synonomous mutations in the human dopamine D2 (DRD2) affect mRNA
35   stability and synthesis of the receptor. Human Molecular Genetics 12:205-216

Gerlach J, 2002 . Improving outcome in schizophrenia: the potential importance of EPS and neuroleptic dysphoria. Ann Clin Psychiatry 14; 47-57

40   Grandy DK, Zhang Y, Civelli O. (1993) PCR detection of the TaqA RFLP at the DRD2 locus. Hum Mol Genet 2, 2197

Grevle L, Guzey C, Hadidi H, Brennersted R, Idle JR, Aasly J. 2000. Allelic association between the DRD2 Taq1 A polymorphism and Parkinson's disease. Mov
45   Disord ;15:1070-4

Heck AH, Haffmans PM, de Groot IW, Hoencamp E. 2000 Risperidone versus haloperidol in psychotic patients with disturbing neuroleptic-induced extrapyramidal symptoms: a double-blind, multi-center trial. Schizophr Res ;46:97-105

5

Hitzemann RJ, (1998) The regulation of D2 dopamine receptor expression. Mol Psychiatry, 3:198-203

Jeste DV, Lacro JP, Bailey A, Rockwell E, Harris MJ, Caligiuri MP. 1999. Lower
10   incidence of tardive dyskinesia with risperidone compared with haloperidol in older patients. J Am Geriatr Soc;47:716-9

Jonsson EG, Nothen MM, Grunhage F, Farde L, Nakashima Y, Propping P, Sedvall GC., (1999) Polymorphisms in the dopamine D2 receptor gene and their relationships
15   to striatal dopamine receptor density of healthy volunteers. Mol Psychiatry , 4:290-6

Kopala LC, 1997. Clinical experience in developing treatment regimens with the novel antipsychotic risperidone. Int Clin Psychopharmacol; 12 Suppl 4:S11-8

20   Laruelle M, Gelernter J, Innis RB (1998) D2 receptors binding potential is not affected by Taq 1 polymorphism at the D2 receptor gene. Mol Psychiatry 3:261-5

Love RC, Nelson MW. 2000. Pharmacology and clinical experience with risperidone. Expert Opin Pharmacother; 1:1441-53
25

Markianos M, Hatzimanolis J, Lykouras L. (2001) Neuroendocrine responsivities of the pituitary dopamine system in male schizophrenic patients during treatment with clozapine, olanzapine, risperidone, sulpiride or haloperidol. Eur Arch Psychiatry Clin Neurosci 251:141-6
30

McCreadie RG, Macdonald IM. (1997) High dosage haloperidol in chronic schizophrenia. Br J Psychiatry ,131:310-6

Mihara K, Kondo T, Suzuki A, Yasui N, Nagashima U, Ono S, Otani K, Kaneko S.
35   (2000) Prolactin response to nemonapride, a selective antagonist for D2 like dopamine receptors, in schizophrenic patients in relation to Taq1 A polymorphism of DRD2 gene. Psychopharmacology (Berl) 149: 246-50

Mihara K, Suzuki A, Kondo T, Yasui-Furukori N, Ono S, Otani K, Kaneko S, Inoue
40   Y. (2001) Relationship between Taq 1A dopamine D2 receptor (DRD2) polymorphism and prolactin response to bromperidol. Am J Med Genet ;105:271-4

Noble EP, Blum K,Ritchie T, Montgomery A, Sheridan PJ .(1991) .Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism.
45   Arch Gen Psychiatry, 48:648-54

Noble EP, Noble RE, Ritchie T, Syndulko K, Bohlman MC, Noble LA, Zhang Y, Sparkes RS, Grandy DK. (1994) D2 dopamine receptor gene and obesity. Int J Eat Disord, 15, 205-17

5

Noble, EP, Zhang, X, Ritchie, T, Lawford B, Grosser, SC, Young, RMcD & Sparkes RS (1998). D2 dopamine receptor and GABA-A receptor $\beta3$ subunit genes and alcoholism. Psychiatry Research, 81, 133-147.

10   Nyberg S, Eriksson B, Oxenstierna G, Halldin C, Farde L .1999. Suggested minimal effective dose based on PET measured D2 and 5-HT2A receptor occupancy in schizophrenic patients. Am J Psychiatry;156:869-75

O'Hara, BF, Smith SS, Byrd G, Persico, AM, Suarez, BK, Catting, GR, Uhl, G (1993).
15   Dopamine D2 receptor RFLPS haplotypes and their association with substance use in black and Caucasian research volunteers. Hum Hered 43: 209-218

Pohjalainen T, Rinne JO, Nagren K, Lehikoinen P, Anttila K, Syvalahti EK, Hietala J.(1998) The A1 allele of the human D2 dopamine receptor gene predicts low D2
20   receptor availability in healthy volunteers. Mol Psychiatry , 3:256-60

Sussman K, 2002. Choosing an atypical antipsychotic. Int Clin Psychopharmacol; 17 Suppl 3:S29-33

25   Tandon R,2002. Safety and tolerability: how do newer generation "atypical" antipsychotics compare? Psychiatr Q ;73:297-311

Thompson J, Thomas N, Singleton A, Piggott M, Lloyd S , Perry EK, Morris CM., Perry RH, Ferrier IN, Court JA.(1997) D2 dopamine receptor gene (DRD2) Taq1 A
30   polymorphism: reduced dopamine receptor binding in the human striatum associated with the A1 allele . Pharmacogenetics ,7:479-84

Wang J Liu ZL, Chen B. 2001. Association study of dopamine D2, D3 receptor gene polymorphisms with motor fluctuations in PD. Neurology;56:1757-9
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Example 2: Antipsychotic-related prolactin elevation is found in Schizophrenics carrying the DRD2 A1 allele.

Hyperprolactinemia induced by antipsychotic medication may be involved in the development of a variety of serious health problems including breast cancer,
40   osteoporosis, cardiovascular disorders and sexual dysfunction. In a sample of 144 schizophrenic patients treated with commonly used antipsychotic medication there was a linear increase in prolactin levels across drug groups reflecting markedly different drug D2 binding affinities. Furthermore, patients with A1+ DRD2 gene status (A1/A1, A1/A2 genotypes) had significantly higher serum prolactin than A1- individuals (A2/A2
45   genotype) (p<0.036) reflecting greater functional D2 drug occupancy in the A1+ group.

Among individuals treated with Clozapine those with the A1+ allele had significantly higher prolactin than their A1- counterparts (p=0.0398). DRD2 variants may be associated with different antipsychotic treatment responses and liability for adverse drug effects. Pharmacogenetic implications of these findings are presented.

5

Introduction

Hyperprolactinemia produced by antipsychotic medication is the result of drug D2 receptor occupancy and consequent blockade of the inhibitory effects of dopamine. (Markianos et. al. 2001). Dopamine is released by the hypothalamus into the

10 hypophyseal portal system and is delivered to the anterior lobe of the pituitary. Dopamine subsequently inhibits prolactin release by binding to D2 receptors. Bound receptors induce changes in membrane channels and G proteins thus suppressing lactotroph prolactin secreting activity. Furthermore, dopamine activates intracellular signalling pathways which decrease gene expression and result in reduced lactotroph

15 proliferation (Iaccarino et. al. 2002). Dopamine release in the hypothalamus is regulated by a variety of mechanisms including oestrogen, thyroid releasing factor, endogenous opioids, psychological and physical stress, neuropeptides, illness (for example, epilepsy and herpes zoster infection), neurotransmitter activity and prolactin itself (Ben-Johnathon & Hnasko, 2001, Petty, 1999).

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Hyperprolactinemia has been considered an inevitable consequence of treatment with any typical antipsychotic agent (Petty, 1999). It may result in depression, sexual dysfunction, amennhorea, galactorrhea, breast cancer and osteoporosis (Halbreich et al 2003). Depression and sexual dysfunction induced by hyperprolactinemia have the

25 potential to adversely influence adherence to treatment (Maguire, 2002) and additional health problems can exacerbate the burden experienced by those with schizophrenia.

Antipsychotics vary widely in their binding affinity for the D2 receptor. Several currently available compounds, such as Clozapine and Quetiapine have a lower D2

30 binding affinity than dopamine (Remington & Kapur 2000) and have not been associated with hyperprolactinemia (Markianos et al 2002). By contrast, hyperprolactinemia is more commonly associated with tighter binding agents such as risperidone and typical antipsychotics (David et al, 2000). Olanzapine, an atypical antipsychotic with intermediate D2 binding affinity has generally been associated with

35 modest increases in prolactin levels (David et al, 2000) and the prolactin levels observed are not an accurate reflection of drug D2 occupancy (Lavalaye et al 1999). In support of this prolactin levels are elevated in patients treated with risperidone, but not when treated with olanzapine, despite comparable D2 drug occupancy levels measured by [123I] iodobenzamide SPECT (Lavalaye et al, 1999).

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Although use of tighter binding agents is generally associated with higher prolactin levels it is a common clinical observation that there are considerable individual variations in prolactin levels induced by identical medication at a given dose. Individual vulnerability factors that are associated with the development of

45 hyperprolactinemia are likely to involve D2 receptor physiology given the central role of

these receptors in prolactin secretion regulation. The A1 allele of the D2 dopamine receptor gene (DRD2) (A1/A1 and A1/A2 genotypes) is associated with significantly reduced density of D2 receptors (Noble et al, 1991). Nemonapride, a selective D2 antagonist, is associated with significantly elevated prolactin in A1+ females (A1/A1 and A1/A2 genotypes) with schizophrenia (Mihara et al 2000). A1+ allelic status is also associated with increased prolactin response to bromperidol in female inpatients with schizophrenia (Mihara et al 2001). In the current study we investigated the possible association of A1+ allelic status with raised prolactin levels in both male and female schizophrenic patients treated with antipsychotics frequently used in clinical practice. The current study investigated the prolactin response to a variety of agents classified as tight (Risperidone, Typicals) intermediate (Olanzapine) or loose (Clozapine) binding (Remington & Kapur, 2000).

Method.

SUBJECTS. One hundred and forty four unrelated Caucasian patients (123 males, 21 females) attending various psychiatric units for the treatment of their schizophrenia were recruited for the study. Nine of the women were post menopausal. All patients met Diagnostic and Statistical Manual of Mental Disorders (DSM-1V) criteria for schizophrenia. They underwent a clinical history taking by either a psychiatrist (SB, BL, MB, WW) or a clinical psychologist (R.McDY). Demographic details including ethnic background data was obtained.

All subjects had received the same antipsychotic medication for at least one month at a stable dose. As other psychoactive agents can influence prolactin levels (Jarvinen et al., 1992, Hugues et al 2000, Keltner et al 2002, Basturk et al 2001) those patients on regular antidepressant, opiate, anxiolytic or mood stabilising medication were excluded from the study. Women on the oral contraceptive pill were excluded. No patients had received prn benzodiazepines for at least 24 hours. Inpatients had not received more than 5mg of prn diazepam equivalent in any 24 hour period during their admission. All patients were physically well and none had a history of epilepsy. The average age of those recruited was 36.41± 12.01 years. At the time of data collection, 61 subjects were inpatients and 83 were outpatients. The breakdown of medications prescribed was as follows: 31 patients were on Clozapine, 31 on Olanzapine, 33 on Typicals (this included 12 patients on Flupenthixol, 2 on Fluphenazine decanoate, 13 on Zuclopenthixol, 3 on Haldon, 1 on Thioridazine, 1 on Thiothixene and 1 on Trufluperazine) and 49 on Risperidone. The dose of the antipsychotics was transformed to Chlorpromazine equivalents per kilograms (CPZEK). The mean mg. CPZEK dose ± SD of each of the four drug groups used was: Clozapine, 5.14 ± 2.94; Olanzapine, 5.37 ± 2.62; Typicals, 5.60 ± 3.70; Risperidone, 4.82 ± 2.00. There were no significant differences in dose used among the four drug groups (F (3,128) = 0.51, P = .67). Weight data was missing on 12 subjects (1 Clozapine patient, 2 Olanzapine patients, 2 Risperidone patients and 7 Typicals patients).

Adherence in inpatients was complete as all subjects were administered medication by nursing staff. Outpatient adherence was estimated by self-report and assessment by the treating psychiatrist. Thirty of the 33 patients receiving typical medication were treated with nurse administered depot preparations.

5

PROCEDURE   Patients were recruited from the Fortitude Valley Community Mental Health Centre, the Royal Brisbane Mental Health Unit and the Park Psychiatric Hospital. These facilities are located in Brisbane, Australia. Inclusion criteria included being aged between 18 and 65 years and having a DSM IV diagnosis of Schizophrenia.

10   As noted all subjects were prescribed the same antipsychotic for at least a month on a stable dose (for at least 2 weeks). Patients taking any regular additional antidepressant, anxiolytic or mood stabilising psychotropic medication were excluded. All subjects had to possess an adequate comprehension of English. Potential participants were excluded if they had any other psychiatric disorder including Schizoaffective Disorder, Bipolar

15   Disorder, Dementia, Organic Brain Syndrome, Major Depressive Disorder with Delusions .

A 10mL blood sample was drawn from each subject for DNA extraction and prolactin determination. DNA was sent to the U.C.L.A. for genotyping. All participants provided

20   informed consent and were able to terminate participation at any time without prejudice. Institutional ethics approval was obtained from the clinics and hospitals involved. Prolactin determination using mIU/l (milli international units per litre) was conducted at the Royal Brisbane Hospital. Prolactin and demographic data were combined with genetic data at the completion of the trial.

25

Genotyping

GENOTYPING

30   . DNA was extracted from leucocytes using standard techniques and subsequently used as a template for determination of Taq1A DRD2 alleles by polymerase chain reaction (Grandy et al 1993) .As previously described (Noble et al 1994) the amplification of DNA was carried out using a Perkin Elmer Gene Amp 9600 thermocycler. Approximately 500ng.of amplified DNA was then digested with 5 units of Taq 1

35   restriction enzyme (GIBCO/BRL, Grand Island, NY) at 65 degrees centigrade overnight. The resulting products were analysed by electrophoresis in a 2.5% agarose gel containing ethidium bromide and visualised under ultraviolet light. The A1/A2 genotype is revealed by three fragments: 310bp, 180bp, and 130bp. A2/A2 genotype is indicated by two fragments: 180bp and 130bp. The A1/A1 genotype is shown by the uncleaved

40   310bp fragment. Subjects with the A1/A1 and A1/A2 genotypes were considered to have the A1+ allelic status, while those with the A2/A2 genotype were considered to have the A1- allelic status.

Data Analysis

Information coded from interview proformas was entered into a computer data base. Chi-square test (Yates corrected) and Fisher's exact test, where appropriate, were employed to compare differences in non continuous variables between A1+ and A1- allelic groups. Analysis of variance (ANOVA) was used to compare differences among the various drug groups in prolactin levels as a continuous variable. Similarly, one-way ANOVA was employed to examine differences in prolactin levels between A1+ and A1- allelic groups. A p-value of <0.05 was considered to be statistically significant.

Results

Consistent with other studies of patients on antipsychotics there was a significant effect for gender with females obtaining significantly higher prolactin elevation than males F $(1,142) = 25.19$, $p < 0.0001$). Males and females were equally represented across the four drug groups. There was also a significant difference in prolactin response among the four antipsychotic treatment groups F $(3,140) = 19.009$, $p < 0.0001$. Figure 1 shows prolactin levels in each of the treatment groups. Olanzapine compared to Clozapine treatment resulted in significantly higher prolactin levels (F $(1, 60) = 4.76$, $P = .033$). Patients treated with Typical antipsychotics had significantly elevated prolactin levels than their Olanzapine treated counterparts ($F(1,62) = 7.60$, $P = .007$. Finally, significantly higher levels of prolactin occurred in patients treated with Risperidone compared to those treated with Typical antipsychotics ($F(1,80) = 8.97$, $P = .004$).

The genotypes of the 144 patients were as follows, A1/A1 (n=7), A1/A2 (n=56) and A2/A2 (n=82) .The age of the A1+ allelic group (35.5 ± 12.7) was not significantly different from that of the A1- allelic group (37.1 ± 11.4) ($F(1,141) = 0.66$, $P = .42$). There were no significant differences in gender ($\chi2$ (1) = 0.048, p =0.83), inpatient or outpatient status ($\chi2$ (1) =0.00, p>.99), family history of schizophrenia ($\chi2(1)$=0.00, p>0.99), criminality ($\chi2(1)$=0.02, p=.90), binge drinking ($\chi2(1)$=0.08, p=.78) or suicide attempts ($\chi2(1)$ = 2.026, p=.16) between A1+ and A1- allelic subjects. There was also no difference in antipsychotic dose between A1 + and A1- allelic groups taking Clozapine ($F(1,28) = 2.36$, $P = .14$), Olanzapine ($F(1,27) = 1.0.$, $P = .33$), Typicals ($F(1,24) = .033$, $P = .69$), and Risperidone ($F(1,45) = .70$, $P = .50$). In terms of clinical status there were no differences in umber of admissions $F(1,140)= 1.447$, p=.230, PANSS positive symptoms $F(1,140) = 0.60$, p=.8061, PANSS Negative symptoms F (1, 139)= 2.604, p=0.1089). .

Table 1 shows serum prolactin levels of A1+ and A1- allelic schizophrenic patients treated with antipsychotic drugs. Using analysis of variance, when all the antipsychotics were considered together patients carrying the A1+ allele had a significant and about a 40% higher Prolactin levels than patients carrying the A1- allele ($F(1,142) = 4.50$, $P = .036$). When individual drug groups were examined, only the loosest binding antipsychotic Clozapine showed a significant and a more than two fold higher Prolactin levels in A1+ compared to A1- allelic patients ($F(1,29) = 4.63$, $P = .04$). To test whether this result was influenced by gender the analysis was rerun for males only as there were

insufficient females to analyse and significance remained $F(1,26) = 4.58$, p=.042). In none of the other three groups of antipsychotics were there significant differences in Prolactin levels between A1+ and A1- allelic patients.

5   Hyperprolactinemia was defined using community sample cut-off levels set at a 95% reference range, 430 mU/l in men and 560 mU/l in women (Vanderpump, French, Appleton, Tunbridge & Taylor, 1998). In total 64 patients, or 44% of the sample, exceeded these levels. Only 7 of these patients were prescribed the low binding agents, Clozapine and Olanzapine confirming that these medications are rarely associated with
10   hyperprolactinemia (5 % o the sample). Disturbingly 40 of the patients on Risperidone exceeded these prolactin levels, indicating that 81 % of patients on this medication were in the hyperprolactinemic range. A Yates corrected chi-square analysis conducted to compare allelic status across the groups with prolactin levels in the normal range and those with hyperprolactinemia was significant ($\chi 2(1) =5.523$, p=0.018).
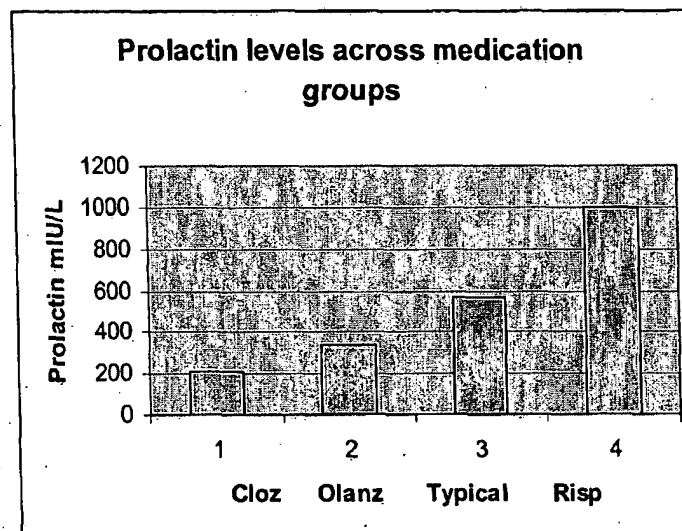
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Table 1: Serum prolactin (in IU/l) means of A1+ and A1- allelic schizophrenics treated with various antipsychotics

GROUP	A1+ Allele	A1- Allele
ALL SUBJECTS, n=144	708 ± 79, n=62	499 ± 62, n=82
CLOZAPINE, n=31	310 ± 92, n=12	147 ± 18, n=19
OLANZAPINE, n=30	327 ± 36, n=9	346 ± 63, n=22
TYPICAL ANTIPSYCHOTICS, n=29	622 ± 94, n=14	531 ± 97, n=19
RISPERIDONE, n=48	1058 ± 142, n=27	928 ± 171, n=22

20

Discussion

Prolactin response to antipsychotic medication was greatest in risperidone followed by typicals, olanzapine and clozapine and was greater in females than males. This
5 increasing order across medications is also found in positron emission tomography (PET) binding studies and confirms identical results described in a previous study (Markianos et al 2001). In that study prolactin response to antipsychotic medication reflected antipsychotic D2 receptor occupancy.

10 Overall, A1+ individuals have significantly higher prolactin levels when treated with a variety of antipsychotic medications. Furthermore, A1+ schizophrenics have significantly higher prolactin levels than A1- patients when treated with the loose binding agent clozapine. The greater prolactin response to antipsychotics witnessed in A1+ schizophrenics in this, and other, (Mihara 2000, 2001) studies indicates that A1+
15 individuals treated with antipsychotic medication generally have greater functional drug D2 occupancy, that is that they have fewer unbound dopamine receptors. Drug D2 binding and individual DRD2 variants are both important factors in determining individual prolactin response to antipsychotic medication. Despite the elevation taking clozapine the majority of the cases with hyperprolactinemia were being administered
20 tight binding agents, there was also a strong allelic effect with A1+ individuals being significantly more likely to be classified as hyperprolactinemic than their A1- counterparts.

There is compelling evidence that A1+ individuals have a reduced density of brain D2
25 dopamine receptors (Noble et al, 1991, Thompson et al, 1997, Pohjalainen et al, 1998, Jonsson, et al, 1999). An early brain autopsy study (Noble et al., 1991) found a significant reduction of approximately 30% in the number of D2 dopamine receptors (Bmax) in the caudate nucleus of A1+ compared to A1- allelic subjects. Moreover, a significant progressive decline in Bmax was found across A2/A2, A1/A2, A1/A1
30 genotypes in that order. There was no difference in D2 dopamine binding affinity (Kd) between A1+ and A1- allelic subjects. Thompson et al., 1997 also reported 30%-40% reduction in D2 dopamine receptor density in the striatum of A1+ individuals compared to A1- individuals. An in-vivo study of healthy Finnish volunteers (Pohjalainen et al., 1998) showed significantly decreased D2 receptor density in the striatum of A1+ when
35 compared with A1- allelic subjects. Again, there was no difference in the Kd between the two groups. In Jonsson et al (1999), another PET study of healthy humans using (11C) raclopride, a significant association of the A1 allele with low D2 dopamine receptor density was found. While, Laruelle et al (1998) reported no association between reduced D2 binding and the A1 allele subjects an editorial (Hitzemann, 1998)
40 suggested the study had insufficient power to detect a significant difference between A1+ and A1- individuals.

The most likely explanation of the association of the A1 allele, a polymorphism in a non-coding region of the DRD2 gene, with reduced D2 receptor density, is that the sequence
45 variation causing the Taq1 A polymorphism is in linkage disequilibrium with functional

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allelic variants that affect receptor expression (Noble, et al, 1998, Comings et al, 1991, O'Hara et al 1993, Arinami et al, 1997). The Taq 1A variants are now known to be in linkage disequilibrium with C957T, a synonymous mutation in the human DRD2. Furthermore, C957T affects mRNA folding leading to both less stable mRNA and
5   decreased translation. These effects dramatically diminish dopamine induced up-regulation of D2 receptors (Duan et al, 2003)..

A1+ individuals with genetically determined reduced D2 density have D2 receptors that are functionally identical to those found in A1- individuals. The binding affinity for
10   dopamine (Kd) is identical in both A1+ and A1- individuals.(Noble et al 1991, Pohjalainen 1998) A1+ individuals will, therefore, at any given dose of antipsychotic medication, tend to have a lower density of free, unbound, D2 receptors .and consequently, greater drug D2 occupancy. This density reflects an active, dynamic, process involving drug displacing dopamine from the receptor and vice versa.
15

D2 receptor-drug occupancy and consequent D2 receptor blockade is necessary for both clinical antipsychotic action (Kapur & Remington, 2001a) and a variety of other effects. Studies with conventional antipsychotics report that approximately 70% occupancy results in maximal therapeutic efficacy (Nordstrom et al 1993). A trend towards
20   improved efficacy in treatment resistant patients has been found when doses of olanzapine were increased to an average of 30.4 mg.(Volavka et al 2002). Preliminary investigations have been undertaken to increase the effectiveness of clozapine, an agent with a high D2 dissociation constant, by adding haloperidol, an agent with a low dissociation constant.(Kapur et al 2001b) A1- individuals are more likely to benefit
25   from these approaches to improve drug D2 receptor occupancy as they have more free, unbound, D2 receptors at any given dose. Conversely A1+ individuals may not derive as much improvement as they have higher functional occupancy. Optimal therapeutic effect is likely to be obtained at lower doses in A1+ schizophrenics .A1- patients may require a higher dose for maximal antipsychotic effect, particularly when prescribed a
30   loose binding antipsychotic such as clozapine or quetiapine. According to the rapid dissociation model (Kapur & Seeman, 2002) atypical agents hypothesised to have an antipsychotic action without causing other effects such as raised prolactin or exrtrapyramidal side effects Kapur & Seeman, 2001). Our data are not consistent with this as even the loosest binding agent Clozapine has other significant effects as
35   illustrated by raised in A1+ individuals compared with those with A1- status, when it acts with the D2 receptor. Antipsychotic as a result of D2 blockade does not occur in isolation of other D2 blockade effects.

40   D2 occupancy also correlates with liability to extrapyramidal adverse effects in patients treated with risperidone (Yamada et al 2002) and a variety of antipsychotics including clozapine (Broich et al 1998) haloperidol.(Kapur et al 2000) and olanzapine (Jauss et al 1998). A1+ individuals treated with antipsychotic medication are likely to experience extrapyramidal adverse effects at lower dose than A1- patients as these patients have
45   decreased nigrostriatal D2 density (Thompson et al, 1997). Future research should

employ this pharmacogenetic approach to investigate clinical parameters in addition to prolactin response. This may result in clinicians having an ability to optimise antipsychotic treatment with regard to drug selection, dose and possible adverse effects.

5

In conclusion, the present study implicates the DRD2 gene as an important determinant of D2 occupancy in schizophrenic patients treated with antipsychotic medications. A1+ patients generally display higher prolactin levels and hence greater functional drug D2 occupancy than A1- individuals. Our results demonstrate that this
10   association is most evident with the loose D2-binding antipsychotic clozapine. . The D2 blocking antipsychotic effect of clozapine may not therefore occur independently of other effects.

15   References

*Arinami T, Gao M, Hamaguchi H, Toru M,(1997) A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. Human Molecular Genetics 6:577.
20
*Basturk M, Karaaslan F, Esel E, Sofuoglu S, Tutus A, Yabanoglu I.(2001) Effects of short and long-term lithium treatment on serum prolactin levels in patients with bipolar affective disorder. Prog Neuropsychopharmacol Biol Psychiatry . 25;315-22

25   *Ben-Jonathon N & Hnasko, R (2001). Dopamine as a prolactin (PRL) inhibitor. Endocr Rev. 6, 724-63.

*Broich K ,Grunwald F, Kasper S, Klemm E, Biersack HJ, Moller HJ. (1998). D2-dopamine receptor occupancy measured by IBZM-SPECT in relation to extra pyramidal
30   side effects. Pharmacopsychiatry: 31;159-62

*Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, Knell E, Kocsis P, Baumgarten R, Kovacs BW, Levy DL, Smith M, Borison RL, Evans DD, Klein DN, MacMurray J, Tosk JF, Sverd J, Gysin R, Flanagan SD,. (1991) The
35   dopamine D2 locus as a modifying gene in neuropsychiatric disorders. Journal of the American Medical Association. 266:1793-1800

David SR , Taylor CC, Kinon BJ, Breier A. (2000) The effects of olanzapine, risperidone, and haloperidol on plasma prolactin levels in patients with schizophrenia.
40   Clin Ther; 9:1085-96.

*Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV (2003) Synonomous mutations in the human dopamine D2 (DRD2) affect mRNA stability and synthesis of the receptor. Human Molecular Genetics 12:205-216
45

*Grandy DK, Zhang Y, Civelli O. (1993) PCR detection of the TaqA RFLP at the DRD2 locus. Hum Mol Genet 2, 2197

*Halbreich U, Kinon BJ, Gilmore JA, Kahn LS. (2003) Elevated prolactin levels in patients with schizophrenia: mechanisms and related adverse effects. Psychoneuroendocrinology, Suppl 1, 53-67.

*Hitzemann RJ, (1998) The regulation of D2 dopamine receptor expression. Mol Psychiatry, 3:198-203

*Hugues FC, Gourlot C, Le Jeunne C. (2000). Drug-induced gynecomastia. Ann Med Interne. 151;10-7

*Iaccarino C, Samad TA, Mathis C, Kercret H, Picetti R, Borrelli E. (2002) Control of lactotrop proliferation by dopamine:essential role of signalling through D2 receptors and ERKs. Proc. Natl. Acad. Sci. ; 99:14530-5

*Jarvinen A, Rago L, Mannisto PT.(1992) Effects of central and peripheral type benzodiazepine ligands on thyrotropin and prolactin secretion . Neuropeptides;21:183-91

*Jonsson EG, Nothen MM, Grunhage F, Farde L, Nakashima Y, Propping P, Sedvall GC., (1999) Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. Mol Psychiatry , 4:290-6

*Jauss M, Schroder J, Pantel J, Bachmann S, Gerdsen I, Mundt C. (1998) Severe akathisia during olanzapine treatment of acute schizophrenia. Pharmacopsychiatry; 4:146-8

*Kapur S, Remington G. (2001a) DopamineD(2) receptors and their role in atypical antipsychotic action: still necessary and may even be sufficient. Biol Psychiatry 50:873-83

*Kapur S, Roy P, Daskalakis J, Remington G, Zipursky RB,. (2001b) Increased dopamine D2 receptor occupancy and elevated prolactin level associated with addition of haloperidol to clozapine. Am J Psychiatry; 159:255-262

*Kapur S, Zipursky R, Jones C, Remington G , Houle S. (2000) Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry ;4:514-20

*Keltner NL, Mcafee KM, Taylor CL.(2002) Mechanisms and treatments of SSRI-induced sexual dysfunction. Perspect Psychiatr Care 38:111-6

*Laruelle M, Gelernter J, Innis RB (1998) D2 receptors binding potential is not affected by Taq 1 polymorphism at the D2 receptor gene. Mol Psychiatry 3:261-5

*Lavalaye J, Linszene DH, Booij J, Reneman L ,Gersons BP, van Royen EA
(1999). Dopamine D2 receptor occupancy by olanzapine or risperidone in young patients with schizophrenia. Psychiatry Research, 8, 33-44.

*Maguire GA (2002). Prolactin elevation with antipsychotic medications: mechanisms of action and clinical consequences. J Clin Psychiatry, 63 Suppl 4, 56-62.

*Markianos M, Hatzimanolis J, Lykouras L. (2001) Neuroendocrine responsivities of the pituitary dopamine system in male schizophrenic patients during treatment with clozapine, olanzapine, risperidone, sulpiride or haloperidol. Eur Arch Psychiatry Clin Neurosci 251:141-6

*Markianos M, Hatzimanolis J, Lykouras L,Christodoulou GN. (2002) Prolactin responses to acute clomipramine and haloperidol of male schizophrenic patients in a drug-free state and after treatment with clozapine or with olanzapine. Schizophrenia Res. ;56:11-7

*Mihara K, Kondo T, Suzuki A, Yasui N, Nagashima U, Ono S, Otani K, Kaneko S. (2000) Prolactin response to nemonapride, a selective antagonist for D2 like dopamine receptors, in schizophrenic patients in relation to Taq1 A polymorphism of DRD2 gene. Psychopharmacology (Berl) 149: 246-50

*Mihara K, Suzuki A, Kondo T, Yasui-Furukori N, Ono S, Otani K, Kaneko S, Inoue Y. (2001) Relationship between Taq 1A dopamine D2 receptor (DRD2) polymorphism and prolactin response to bromperidol. Am J Med Genet ;105:271-4

*Noble EP, Blum K,Ritchie T, Montgomery A, Sheridan PJ .(1991) .Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. Arch Gen Psychiatry, 48:648-54

*Noble EP, Noble RE, Ritchie T, Syndulko K, Bohlman MC, Noble LA, Zhang Y, Sparkes RS, Grandy DK. (1994) D2 dopamine receptor gene and obesity. Int J Eat Disord, 15, 205-17

*Noble, EP, Zhang, X, Ritchie, T, Lawford B, Grosser, SC, Young, RMcD & Sparkes RS (1998). D2 dopamine receptor and GABA-A receptor β3 subunit genes and alcoholism. Psychiatry Research, 81, 133-147.

*Nordstrom AL, Farde L, Wiesel FA, Forslund K, Pauli, S, & Halldin, C, & Uppfeldt, G (1993). Central D2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double blind PET study of schizophrenic patients. Biol Psychiatry:33: 227-235

*O'Hara, BF, Smith SS, Byrd G, Persico, AM, Suarez, BK, Catting, GR, Uhl, G (1993). Dopamine D2 receptor RFLPS haplotypes and their association with substance use in black and Caucasian research volunteers. Hum Hered 43: 209-218.

*Petty RG .(1999) Prolactin and antipsychotic medications: mechanism of action. Schizophr Res 35 Suppl :S67-73 .

*Pohjalainen T, Rinne JO, Nagren K, Lehikoinen P, Anttila K, Syvalahti EK, Hietala J.(1998) The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. Mol Psychiatry , 3:256-60

*Remington G, Kapur S, (2000) Atypical antipsychotics: are some more atypical than others? Psychopharmacology(Berlin); 148:3-15.

*Thompson J, Thomas Perry RH, Ferrier IN, Court JA.(1997) D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine receptor binding in the human striatum associated N, Singleton A, Piggott M, Lloyd S, Perry EK, Morris CM, with the A1 allele., Pharmacogenetics ,7:479-84

Vanderpump M, French, Appleton, Tunbridge & Taylor, (1998). The prevealence of hyperprolactinemia and association with markers of autoimmune thyroid disease in survivors of the Whickham Survey cohort. Clinical Endocrinology, 48, 1365-2265.

*Volavka, J, Czobor, P, Shieitman, B, Lindenmayer, JP, Citrome, L, McEvoy, JP, Cooper, TB, Chakos, M & Lieberman, JA (2002). Clozapine, olanzapine, ripseridone, and haloperidol in the treatment of patients with chronic schizophrenia and schizoaffective disorder. Am J Psychiatry, 159, 255-62.

*Yamada Y, Ohno Y, Nakashima Y, Fukuda M, Takayanagi R, Sato H, Sawada Y, Iga T. (2002) Prediction and assessment of extrapyramidal side effects induced by risperidone based on dopamine D(2) receptor occupancy. Synapse: 46;32-7

Example 3: D2 Dopamine Receptor Gene Polymorphism Differentiates Paroxetine Treatment Outcome in Posttraumatic Stress Disorder

To determine whether allelic status of the D2 dopamine receptor (DRD2) gene differentiates response to a selective serotonin reuptake inhibitor in the treatment of posttraumatic stress disorder (PTSD). Sixty-five Caucasian war veterans with combat-related PTSD were treated with paroxetine for 8 weeks. Patients were assessed at baseline and at follow-up using the General Health Questionnaire-28 (GHQ). TaqI A

DRD2 alleles were determined by PCR. There was a trend for more DRD2 A1- (A2A2)

than A1+ (A1A1) allelic patients to discontinue treatment due to adverse events. In the

remaining 45 patients, significant improvement ($P = 0.015$) in the GHQ total score

occurred over the treatment period. However, when patients were classified by their

5   allelic status, GHQ total score of A1+ patients showed a significant positive treatment

response ($P = 0.010$), whereas the A1- patients did not. Similarly, a significant positive

outcome was found in three of the four GHQ subscale scores in A1+ compared to A1-

patients. These included anxiety/insomnia ($P = 0.013$), social dysfunction ($P = 0.005$),

and depression ($P = 0.016$). DRD2 variants predict response to paroxetine in PTSD.

10   The study suggests a pharmacogenetic approach to the treatment of this disorder.

1. Introduction

Combat-related posttraumatic stress disorder (PTSD) is a highly debilitating

condition with a chronic course. The quality of life of PTSD patients is frequently

15   compromised by comorbid conditions such as social anxiety disorder, panic disorder,

generalized anxiety disorder, dysthymia and major depressive disorder (Zatzick et al.,

1997; O'Toole et al., 1998). Selective serotonin reuptake inhibitors (SSRIs) are an

effective treatment for a wide variety of psychiatric disorders. These include PTSD,

depression, social phobia, and mixed anxiety and depressive states. Indeed, SSRIs are

20   generally accepted to be the first line pharmacotherapy for PTSD (Hidalgo and

Davidson, 2000; Ballinger et al., 2000).

SSRI treatment outcomes are variable. A recent randomized controlled trial of

the SSRI sertraline in the treatment of PTSD revealed a responder rate of 53% compared

to 32% with placebo (Brady et al., 2000). Another study reported that only 50% of

chronic PTSD patients experienced substantial improvement in their symptoms with

antidepressant treatment (Dow and Kline, 1997). Studies in social phobia, major

depressive disorder and dysthymia indicate similar SSRI response rates of 55% (Stein et

5   al., 1998), 69% (Van Houdenhove et al., 1997) and 59% (Thase et al., 1996)

respectively. Individuals across diverse diagnostic groups show similar SSRI response

rates of approximately 20% greater than placebo and 30-50% of patients do not respond

to SSRI medication. Furthermore, the rates of response to different SSRIs are not

significantly different despite their varying pharmacological profiles (Nelson, 1999).

10   Moreover, few patient predictors of response have been identified to assist the clinician

in the selection of an antidepressant (Nelson, 1999).

The monoamine deficiency hypothesis (Delgado, 2000) predicts that depression

15   is a consequence of depleted central nervous system monoamine (noradenaline,

serotonin and dopamine) levels. This hypothesis is supported by the observation that

antidepressants increase the levels of various brain monoamines. However, despite

much research attention, a primary dysfunction has not been found in the brain

monoamine systems of patients suffering from depressive disorders (Delgado, 2000).

20   Regarding PTSD, dopaminergic tone is diminished in response to stress which results in

a hypodopaminergic state amongst sufferers (Deutch and Roth, 1990; Deutch and

Young, 1995). SSRIs have a significant impact on dopaminergic function, with

stimulation of 5-HT 1A and 5-HT 1B receptors facilitating dopamine release and

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stimulation of the 5-HT 2 receptor inhibiting dopamine release (Ng et al., 1999; Rollema et al., 2000; Gobert et al., 2000). In vivo brain microdialysis studies indicate that the SSRIs fluoxetine and fluvoxamine increase dopamine efflux (Mendlin et al., 1998).

5   The mesolimbic dopamine system has been implicated in rapid antidepressant action (Wilner, 1997). D2 dopamine receptors, are particularly important in this antidepressant therapeutic action. Interestingly, paroxetine's antidepressant response is greater in subjects with low D2 dopamine responsivity (Healey and McKeon, 2000). Moreover, striatal D2 dopamine receptor binding, measured by (^{123}I) IBZM, using single

10   photon emission computed tomography, is lower at baseline in SSRI treatment responders than in depressed non-responders and non-depressed controls (Klimke et al., 1999). Furthermore, increases in D2 dopamine receptor binding over time were found in treatment responders, and conversely, treatment non-responders showed decreases in D2 dopamine receptor binding (Klimke et al., 1999).

15   Given the association of brain D2 dopamine receptor levels and responsivity to SSRIs, an investigation of variants of the D2 dopamine receptor (DRD2) gene on SSRI treatment outcome is warranted. An *in vitro* study using (^{3}H)spiperone (Noble et al., 1991) as a D2 dopamine receptor ligand, found a significant decrease in the number of D2 dopamine receptors in the brains of subjects with the DRD2 A1+ (A1A1 and A1A2

20   genotypes) allele than in those with the A1- (A2A2 genotype) allele. An autoradiographic study (Thompson et al., 1997) using (^{3}H)raclopride as a D2 dopamine receptor ligand found significantly reduced D2 dopamine receptor binding in the brains of A1+ compared with A1- allelic subjects. An in vivo positron emission tomography

(PET) study, using (^{11}C)raclopride, also found a significant reduction in brain D2

dopamine receptor density in healthy subjects with the A1+ allele (Pohjalainen et al.,

1998). Moreover, a more recent PET study of healthy humans (Jönsson et al., 1999),

again using (^{11}C)raclopride, found a significant association of the A1 allele with

5   measures of low D2 dopamine receptor density. Another study (Laruelle et al., 1998)

determined in healthy controls and in schizophrenics D2 dopamine receptor binding

potential using (^{123}I) IBZM. No significant difference in this combined sample was

found in the D2 dopamine receptor binding potential between A1+ and A1- allelic

subjects. However, when the controls and schizophrenics were separately examined, a

10   trend for a lower binding potential was found in A1+ allelic controls, whilst a trend for a

higher binding potential was noted in A1+ allelic schizophrenics when compared to

their respective A1- allelic subjects. Since two of the above studies (Pohjalainen et al.,

1998; Laruelle et al., 1998) appeared in the same journal issue, an editorial (Hitzemann,

1998) reviewed their merits. It suggests that the study using (^{123}I) IBZM (Laruelle et al.,

15   1998) had insufficient power to detect a significant difference between A1+ and A1-

allelic controls. Moreover, since schizophrenics showed a trend in the opposite

direction, the results on D2 dopamine receptor binding potential and allelic association

in these subjects may have been confounded by prior neuroleptic treatment. Indeed, in a

recent PET study (Silvestri et al., 2000) using (^{11}C)raclopride, increased D2 dopamine

20   receptor binding was shown in schizophrenic patients subsequent to neuroleptic

treatment.

If variants of the DRD2 gene are associated with lower number of D2 dopamine

receptors, and if SSRI treatment is more efficacious in subjects with decreased D2

dopamine receptor binding, would variants of the DRD2 gene predict SSRI response?

In the present study, behavioral outcome based on TaqI A DRD2 variants was

ascertained in PTSD patients treated with paroxetine.

2. Materials and methods

5   Sixty-five unrelated male Caucasian patients with the diagnosis of PTSD were

recruited for study. All subjects were Vietnam combat veterans who had served in the

Australian armed forces. None were being treated with psychotropic medication.

Patients were excluded from the study if they had a diagnosis of psychosis, bipolar

disorder, obsessive compulsive disorder, organic brain syndrome, glaucoma, cardiac

10   disease, or were being treated with anticoagulants or drugs affecting hepatic metabolism

(Aropax® [paroxetine] product information, 1999).

          Patients were assessed for PTSD by a consultant psychiatrist (BL) or a

psychiatric registrar (BK) using Diagnostic and Statistical Manual of Mental Disorders

(DSM-IV) criteria. PTSD caseness was confirmed by all patients exceeding the clinical

15   cut-off score of 94 on the Mississippi Scale for combat-related posttraumatic stress

disorder (Zatzick et al., 1997). The validity and reliability of the Mississippi scale are

well established in veterans (Keane et al., 1988). Patients then underwent clinical

history taking by a psychiatrist or by a clinical nurse (EP). Demographic data and ethnic

background were also obtained.

20   After initial assessment, patients were medically examined and then were started

on 20 mg/day of paroxetine for the first two weeks followed by a 40 mg/day dose for the

remaining six weeks. It should be noted that response to this drug commences after one

week of treatment but does not exceed placebo until the second week of therapy (Aropax® [paroxetine] product information, 1999).

The General Health Questionnaire–28 (GHQ) was administered at baseline (0-7 days on paroxetine) and at the end of treatment (8 weeks after commencement on

5   paroxetine). This questionnaire measures four psychopathological factors particularly relevant to PTSD and its comorbid psychiatric conditions: somatic concerns (GHQ1), anxiety/insomnia (GHQ2), social dysfunction (GHQ3) and depression (GHQ4). The GHQ has been widely validated internationally as a means of detecting psychiatric caseness and is sensitive to change (Ormel et al., 1989). The GHQ has utility as a

10 follow-up measure of veteran mental health following exposure to combat (Deahl et al., 1994) and is sensitive to changes in combat-related PTSD symptoms (Ward, 1997).

A 10ml blood sample was drawn from each patient. Genomic DNA was extracted employing standard techniques and used as a template for determination of TaqI A DRD2 alleles by the polymerase chain reaction (Grandy et al., 1993). The

15 amplification of DNA was carried out using a Perkin Elmer GeneAmp 9600 thermocycler. Approximately 500 ng of amplified DNA was digested with five units of TaqI restriction enzyme (New England Biolabs) at 65°C overnight. The resulting products were separated by electrophoresis in a 2.5% agarose gel containing ethidium bromide and visualized under ultraviolet light. Three genotypes are obtained: the A1A2

20 genotype is revealed by three fragments: 310 bp, 180 bp and 130 bp. The A2A2 genotype by two fragments: 180 bp and 130 bp. The A1A1 genotype is shown by the uncleaved 310 bp fragment. A1+ allelic subjects are those that either have the A1A1 or A1A2 genotype; A1- allelic subjects have the A2A2 genotype only.

Patient assessments were conducted blind to their DRD2 allelic status. In
addition to their standard treatment, patients were monitored twice for adverse events.
Drug compliance was checked by pill count. All participants provided written informed
consent and were able to terminate treatment without prejudice. However, those
5   terminating participation were asked to provide reasons for their withdrawal.
Institutional ethics approval was obtained from the Greenslopes Private Hospital.

Information coded from the interviews, GHQ and genotyping results were
entered into a computer database. Nominal data were analyzed by Yates corrected X^2
test and continuous data, with repeated measures, were analyzed by paired t-test or by
10   analysis of variance. P values ≤ 0.05 were considered statistically significant. P values
≥ 0.05 but < 0.10 were considered to be approaching a significant level.

3. Results

The mean age $\pm$ S.E. of the 65 patients who entered treatment was 51.4 ± 0.7
years. They had the following genotypes: A1A2 (A1+ allele), $n = 26$; and A2A2 (A1-
15   allele), $n = 39$. This genotype distribution did not deviate from Hardy-Weinberg
equilibrium ($\chi^2 = 2.86$, $P = 0.091$). There was no significant difference in the ages of
A1+ (51.1 ± 0.7 years) and A1- (51.6 ± 1.0 years) allelic subjects ($F(1,63) = 0.13$, $P =$
0.72).

Of the 65 patients who entered the study, 20 (4 A1+ and 16 A1-) discontinued
20   paroxetine treatment for a variety of reasons. A trend ($P = 0.055$) was found in the
greater number of A1- than A1+ allelic subjects who discontinued treatment. In the
group that discontinued treatment, 2 A1+ and 11 A1- allelic subjects had adverse events

28

relating to anxiety, insomnia, headache or tremor. In addition, 2 A1+ and 5 A1- allelic

subjects experienced erectile dysfunction, decreased libido or delayed ejaculation.

The 45 subjects who completed treatment were 51.8 ± 0.8 years old. They had

the following alleles: A1+, $n = 22$; A1-, $n = 23$. There was no significant difference in

5   the ages of these A1+ (51.4 ± 0.9 years) and A1- (52.2 ± 1.4 years) allelic subjects

$(F(1,43) = 0.27, P = 0.61)$.

As indicated earlier, 20 of the initial subjects dropped out of the study for a

variety of reasons. The baseline GHQ total score of these subjects was 44.8 ± 3.7. In

the remaining 45 subjects the GHQ total score was 48.0 ± 2.5. There was no significant

10   difference in the GHQ total score between those who dropped out and those who

remained in the study $(F(1,63) = 0.55, P = 0.46)$.

Table 1 shows the baseline GHQ total and subscale scores of the 65 patients who

entered the paroxetine treatment study based on the presence or absence of the DRD2

A1 allele. GHQ total score was significantly higher in A1+ compared to A1- allelic

15   patients. No significant difference was found in GHQ1 (somatic concerns) subscale

score between these two allelic groups. However, GHQ2 (anxiety/insomnia), GHQ3

(social dysfunction) and GHQ4 (depression) subscale scores were significantly higher in

A1+ allelic subjects when compared to their respective A1- allelic counterparts.

Figure 1 shows the baseline and treatment GHQ total scores of the 45 patients

20   who remained in the study. A significant improvement was found in the total patients

during the course of paroxetine treatment $(t = 2.5, P = 0.015)$. Figure 1 also presents the

baseline and treatment GHQ total scores of those patients based on their allelic status.

The results showed a significant improvement in the GHQ total score in the patients

with the A1+ allele ($t = 2.8$, $P = 0.010$), but not in those with the A1- allele ($t = 0.99$, $P = 0.334$).

Table 2 presents the results of the four GHQ subscale scores at baseline and at treatment of the 45 patients who remained in treatment, based on their allelic status. In

5 the total patient group, GHQ1 subscale score was significantly reduced over the course of treatment. A trend toward significant improvement was found in the A1+ allelic subgroup. GHQ2 subscale score was significantly reduced in the total patient group over the course of treatment. However, this significant reduction was found only in the A1+ allelic subgroup. There was a trend toward a significant improvement in GHQ3

10 subscale score in the total patient group over the course of treatment, with A1+ allelic subjects experiencing a significant improvement. In contrast, A1- allelic subjects showed no such improvement. Finally, GHQ4 subscale score revealed a significant improvement in the total patient group during treatment, with A1+ allelic subjects again showing a significant improvement, while A1- allelic subjects showing no significant

15 effect.

Whereas, as shown in Table 1, significantly higher baseline GHQ total score and its three subscale scores (GHQ2, GHQ3, GHQ4) were found in the A1+ compared to the A1- allelic groups, at the end of Paroxetine treatment, no significant differences were found between these allelic groups in any of the GHQ scores measured: GHQ total

20 ($F(1,43) = 0.001$, $P = 0.99$); GHQ1 ($F(1,43) = 1.5$, $P = 0.23$); GHQ2 ($F(1,43) = 0.14$, $P = 0.71$); GHQ3 ($F(1,43) = 1.1$, $P = 0.30$); GHQ4 ($F(1,43) = 2.5$, $P = 0.12$).

4. Discussion

In the present study, and consistent with previous studies (Marshall et al., 1998; Zygmont et al., 1998), paroxetine treatment was effective in reducing psychopathological symptoms in Vietnam veterans with combat-related PTSD. However, this reduction in total symptoms measured was dependent on the patients'

5   DRD2 allelic status, with DRD2 A1 allelic patients showing a significant reduction, while no such reduction was found in patients without this allele.

At baseline, total psychopathology score was greater in patients with than in patients without the DRD2 A1 allele. This initial greater total symptoms score in DRD2 A1 allelic patients was also evident in the subscale scores of anxiety/insomnia, social

10   dysfunction and depression. However, after 8 weeks of paroxetine treatment, these subscale scores, like the total psychopathology score, were more significantly reduced in patients who carried the DRD2 A1 allele than those who did not. This resulted in no significant differences at the end of treatment between these two allelic groups in any of the GHQ scores measured.

15   Why do PTSD patients with the DRD2 A1 allele receive the greatest benefit after treatment with paroxetine? The answer to this question remains unknown. However, it may be hypothesized that baseline brain D2 dopamine receptor density could differentially affect subjects' drug response. In support of this hypothesis are studies which showed that paroxetine's antidepressant response is greater in subjects

20   with low D2 dopamine receptor responsivity than subjects with high responsivity (Healey and McKeon, 2000). Moreover, D2 dopamine receptor binding is lower at baseline in SSRI treatment depressed responders than in depressed non-responders (Klimke et al., 1999). This differential response extends to another drug (Volkow et al.,

1999). In that PET study, the response to methylphenidate, a psychostimulant, was found to be pleasant and positively reinforcing in subjects with low brain D2 dopamine receptor levels, whereas those with high D2 dopamine receptor levels found this drug to be aversive.

5   Growing evidence (Noble et al., 1991; Thompson et al., 1997; Pohjalainen et al., 1998; Jönsson et al., 1999) suggests that individuals with the DRD2 A1 allele have reduced number of brain D2 dopamine receptors. In a double blind bromocriptine (a D2 dopamine receptor agonist) –placebo study of alcoholics (Lawford et al., 1995), the greatest decrease in anxiety and craving as well as the best retention rate was found in

10   DRD2 A1 allelic patients who received bromocriptine. This pharmacogenetic approach to treatment is similar to the present study where paroxetine treatment was found to be most effective in reducing psychopathological symptoms in patients with the DRD2 A1 allele. Moreover, albeit not achieving a significant level, patient retention rate was greater (due to fewer adverse events) in patients who carried than those who did not

15   carry the DRD2 A1 allele.

The reason why subjects with low D2 dopamine receptor binding respond to SSRIs remains unknown. However therapeutic actions of drugs and their adverse effects are thought to be due to neurotransmitter activating genes in target neurons (Stahl, 1999). Fluoxetine causes an increase in nucleus accumbens shell D2 dopamine

20   receptor mRNA resulting in increased D2 dopamine receptor postsynaptic binding in the nucleus accumbens (Ainsworth et al., 1998). Similarly, the SSRI citalopram increases D2 dopamine receptor mRNA in the striatum and nucleus accumbens (Dziedzicka-Wasylewska et al., 1997). This effect of citalopram on increased transcription of the D2

dopamine receptor gene is likely to be mediated by increased serotonin levels induced

by SSRIs, as 5 hydroxytryptophan also increases D2 dopamine receptor mRNA

transcription (Kameda et al., 2000). Induction of D2 dopamine receptor gene

expression may be the mechanism by which SSRIs reduce both anxiety and depression.

5   Low pretreatment D2 dopamine receptor density may be a requirement for effective

treatment. Conversely high pretreatment D2 dopamine receptor density may result in

treatment resistance.

SSRI medications have similar efficacy in the treatment of depression and

anxiety disorders. There is a positive response to SSRIs in subjects who have anxiety

10   disorders without depression (de Boer et al. 1995), depression without anxiety (Nelson,

1999) and mixed anxiety and depressive disorder (Kara et al., 1994). There is also a

significant association between anxiety and depressive scores in normal subjects and in

patients with anxiety and depressive disorders (Kaneda and Fujii, 2000). With regard to

genotypic structure, there is considerable evidence that anxiety and depression share a

15   common genetic diathesis (Mineka et al., 1998). That common genetic diathesis may be

the DRD2 gene is supported by the association of DRD2 variants with anxiety and

depression, irrespective of the clinical disorder studied (Peroutka et al., 1998;

Samochowiez et al., 2000). The impact of Paroxetine on both anxiety and depression

scores in DRD2 A1 allelic subjects of the present study further argues for the

20   interrelated nature of anxiety and depressive disorders in these PTSD patients.

There are several limitations to this preliminary study. The study is an open

label trial and hence the influence of both patient and clinician expectancy cannot be

controlled. However, as both patient and clinician were blind to the patient's DRD2

allelic status, the results are unlikely to be biased by expectancy effects. The sample

size is modest, however, the significance of the results indicates a large effect size. The

subjects were all males and the relevance of the findings to females remains unknown.

Given these limitations, it is recommended that future studies employ double blind

5   paroxetine–placebo trials using a larger number of patients than herein and consisting of

both males and females.

In sum, PTSD patients with the DRD2 A1 allele, in contrast to those without this

allele, showed a significant positive response to paroxetine treatment. The study

suggests a pharmacogenetic approach to the treatment of PTSD.

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References

Ainsworth, K., Smith, S.E., Zetterstrom, T.S., Pei, Q., Franklin, M., Sharp, T., 1998.

Effect of antidepressant drugs on dopamine D1 and D2 receptor expression and

dopamine release in the nucleus accumbens of the rat. Psychopharmacology 140,

15   470-477.

Aropax® (Paroxetine) product information, 1999. SmithKline Beecham (Aust) Pty Ltd,

Item number 92536.

Ballinger, J.C., Davidson, J.R., Lecrubier, Y., Nutt, D.J., Foa, E.B., Kessler, R.C.,

Macfarlane, A.C., Shalev, J.C., 2000. Consensus statement on Posttraumatic Stress

20   Disorder from the International Consensus Group on Depression and anxiety. J.

Clin. Psychiatry 61, Supp 5, 60-66.

Brady, K., Pearlstein, T., Asnis, G.M., Baker, D., Rothbaum, B., Sikes, C.R., Farfel,

G.M., 2000. Efficacy and safety of sertraline treatment of posttraumatic stress

disorder; a randomized controlled trial. JAMA 12:283, 1837-1844.

Deahl, M.P., Gillham, A.B., Thomas, J., Searle, M.M., Srinivasin, M., 1994.

5   Psychological sequelae following the Gulf War. Factors associated with subsequent

morbidity and the effectiveness of psychological debriefing. Br. J. Psychiatry 165,

60-65.

Delgado, P.L., 2000. Depression: the case for a monoamine deficiency. J. Clin.

Psychiatry, 61 Suppl 6, 7-11.

10   den Boer, J.A., Westenberg, H.G., de Leceeun, A.S., van Vliet, I.M., 1995. Biological

dissection of anxiety disorders: the clinical role of selective serotonin reuptake

inhibitors with particular reference to fluvoxamine. Int. Clin. Psychopharmacol.

Suppl 4, 47-52.

Deutch, A.Y., Roth, R.H., 1990. The determinants of stress-induced activation of the

15   prefrontal cortical dopamine system. Prog. Brain Res. 85, 357-393.

Deutch, A.Y., Young, C.D., 1995. A model of the stress-induced activation of prefrontal

cortical dopamine systems: coping and the development of post-traumatic stress

disorder. In:, Friedman, M.J., Charney, D.S., Deutch, A.Y. (Eds.), Neurobiological

and Clinical Consequences of Stress. Lippincott-Raven Press, Philadelphia, pp. 163-

20   176.

Dow, B., Kline, N., 1997. Antidepressant treatment of posttraumatic stress disorder and

major depression in Veterans. Ann. Clin. Psychiatry 9, 1-5.

Dziedzicka-Wasylewska, M., Rogoz, R., Klimek, V., Maj, J., 1997. Repeated

administration of antidepressant drugs affects the levels of mRNA coding for D1

and D2 dopamine receptors in the rat brain. J. Neural. Transm. 10, 515-524.

Gobert, A., Rivet, J.M., Lejeune, F., Newman-Tancredi, A., Adhumeau-Auclair, A.,

5   Nicolas, J.P., Cistarelli, L., Melon, C., Millan, M.J., 2000. Serotonin (2C) receptors

tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not

serotonergic, pathways: a combined dialysis and electrophysiological analysis in the

rat. Synapse 36, 205-221.

Grandy, D.K., Zhang, Y., Civelli, O., 1993. PCR detection of the Taq A RFLP at the

10   DRD2 locus. Hum. Mol. Genet. 2, 2197.

Healy, E., McKeon, P., 2000. Dopaminergic sensitivity and prediction of antidepressant

response. J. Psychopharmacology 14, 152-156.

Hidalgo, R.B., Davidson, J.R.T., 2000. Selective serotonin reuptake inhibitors in post-

traumatic stress disorder. J. Psychopharmacol. 14, 70-76.

15   Hitzemann, R.J., 1998. The regulation of D$_2$ dopamine receptor expression. Mol.

Psychiatry 3, 198-203.

Jönsson, E.G., Nöthen, M.M., Grünhage, F., Farde, L., Nakashima, Y., Propping, P.,

Sedvall, G.C., 1999. Polymorphisms in the dopamine D2 receptor gene and their

relationships to striatal dopamine receptor density of healthy volunteers. Mol.

20   Psychiatry 4, 290-296.

Kameda, K., Kusumi, I., Suzuki, K., Miura, J., Sasaki, Y., Koyama, T., 2000. Effects of

citalopram on dopamine D2 receptor expression in the rat brain striatum. J. Mol.

Neurosci. 14, 77-86.

36

Kaneda, Y., Fujii, A., 2000. Relation between anxiety and depressive symptoms in

      normal subjects and patients with anxiety and/or mood disorders. J. Med. Invest.

      47, 14-18.

Kara, S., Yazici, K.M., Gülec, C., Ünsal, I., 2000. Mixed anxiety-depressive disorder

5   and major depressive disorder: comparison of the severity of illness and

      biological variables. Psychiatry Res. 94, 59-66.

Keane, T.M., Caddell, J.M., Taylor, K.L., 1988. Mississippi Scale for combat-related

      posttraumatic stress disorder: three studies in reliability and validity. J. Consult.

      Clin. Psychol. 56, 85-90.

10   Klimke, A., Larisch, R., Janz, A., Vosberg, H., Muller-Gartner, H.W., Gaebel, W.,

      1999. Dopamine D2 receptor binding before and after treatment of major

      depression measured by [^{123}I] IBZM SPECT. Psychiatry Res. Neuroimaging. 90,

      91-101.

Laruelle, M., Gelernter, J., Innis, R.B., 1998. D_2 receptors binding potential is not

15   affected by TaqI polymorphism at the D_2 receptor gene. Mol. Psychiatry 3, 261-

      265.

Lawford, B.R., Young, R.McD., Rowell, J.A., Qualichefski, J., Fletcher, B.H.,

      Syndulko, K., Ritchie, T., Noble, E.P., 1995. Bromocriptine in the treatment of

      alcoholics with the D2 dopamine receptor A1 allele. Nature Med. 1, 337-341.

20   Marshall, R.D., Schneier, F.R., Fallon, B.A., Knight, C.B., Abbate, L.A., Goetz, P.,

      Campeas, R., Liebowitz, M.R., 1998. An open trial of paroxetine in patients

      with noncombat-related chronic posttraumatic stress disorder. J. Clin.

      Psychopharmacol. 18, 10-18.

Mendlin, A., Martin, F.J., Jacobs, B.L., 1998. Involvement of dopamine D2 receptors in

apomorphine induced facilitation of forebrain serotonin output. Eur. J.

Pharmacol. 351, 291-298.

Mineka, S., Watson, D., Clark, L.A., 1998. Comorbidity of anxiety and unipolar mood

5   disorders. Annu. Rev. Psychol. 49, 377-412.

Nelson, J.C., 1999. A review of the efficacy of serotonergic and noradrenergic reuptake

inhibitors for treatment of major depression. Biol. Psychiatry 46, 1301-1308.

Ng, N.-K., Lee, H.-S., Wong, P.T.H., 1999. Regulation of striatal dopamine release

through 5-HT$_1$ and 5-HT$_2$ receptors. J. Neurosci. Res. 55, 600-607.

10   Noble, E.P., Blum, K., Ritchie, T., Montgomery, A., Sheridan, P.J., 1991. Allelic

association of the D2 dopamine receptor gene with receptor binding

characteristics in alcoholism. Arch. Gen. Psychiatry, 48, 648-654.

Ormel, J., Koeter, M.W., van der Brink, W., Giel, R., 1989. Concurrent validity of

GHQ-28 and PSE as measures of change. Psychol. Med., 19, 1007-1013.

15   O'Toole, B.I., Marshall, R.P., Schureck, R.J., Dobson, M., 1998. Posttraumatic stress

disorder and comorbidity in Australian Vietnam veterans: risk factors, chronicity

and combat. Aust. N. Z. J. Psychiatry, 32, 32-42.

Pohjalainen, T., Rinne Peroutka, S.J., Price, S.C., Wilhoit, T.L., Jones, K.W., 1998.

Comorbid migraine with aura, anxiety, and depression is associated with

20   dopamine D2 receptor (DRD2) *Nco*I alleles. Mol. Medicine 4, 14-21.

, J.O., Någren, K., Lehikoinen, P., Anttila, K., Syvalahti, E.K.G., Hietala, J., 1998. The

A1 allele of the human D$_2$ dopamine receptor gene predicts low D$_2$ receptor

availability in healthy volunteers. Mol. Psychiatry 3, 256-260.

Rollema, H., Lu, Y., Schmidt, A.W., Sprouse, J.S., Zorn, S.H., 2000. 5-HT (1A)

receptor activation contributes to ziprasidone induced dopamine release in the

rat prefrontal cortex. Biol. Psychiatry 48, 229-237.

Samochowiec, J., Ladehoff, M., Pelz, J., Smolka, M., Schmidt, L.G., Rommelspacher,

5   H., Finckh, U., 2000. Predominant influence of the 3'-region of dopamine D2

receptor gene (DRD2) on the clinical phenotype in German alcoholics.

Pharmacogenetics 10, 471-475.

Silvestri, S., Seeman, M.V., Negrete, J.-C., Houle, S., Shammi, C.M., Remington, G.J.,

Kapur, S., Zipursky, R.B., Wilson, A.A., Christensen, B.K., Seeman, P., 2000.

10   Increased dopamine D_2 receptor binding after long–term treatment with

antipsychotics in humans: a clinical PET study. Psychopharmacology 152, 174-

180.

Stahl, S.M., 1999. Molecular neurobiology for practicing psychiatrists, part 4:

transferring the message of chemical neurotransmission from presynaptic

15   neurotransmitter to postsynaptic gene expression. J. Clin. Psychiatry 60, 813-

814.

Stein, M.B., Liebowitz, M.R., Lydiard, R.B., Pitts, C.D., Bushnell, W., Gergel, I., 1998.

Paroxetine treatment of generalized social phobia (social anxiety disorder): a

randomized controlled trial. JAMA 280, 708-713.

20   Thase, M.E., Fava, M., Halbreich, U., Cocsis, J.H., Coran, L., Davidson, J., Rosenbaum,

J., Harrison, W., 1996. A placebo controlled, randomized clinical trial

comparing sertraline and imipramine for the treatment of dysthymia. Arch. Gen.

Psychiatry 53, 777-784.

Thompson, J., Thomas, N., Singleton, A., Piggott, M., Lloyd, S., Perry, E.K., Morris, C.M., Perry, R.H., Ferrier, I.N., Court, J.A., 1997. D2 dopamine receptor gene (DRD2) TaqI A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. Pharmacogenetics 7, 479-484.

5   Van Houdenhove, B., Onghena P., Floris, M., Janssen, F., De Nayer, A.R., Boxus, A., Iancu, H., 1997. An open study of sertraline in acute and continuation treatment of depressed out-patients. J. Int. Med. Res. 25, 340-353.

Volkow, N.D., Wang, G.J., Fowler, J.S., Logan, J., Gatley, S.J., Gifford, A., Hitzemann, R., Ding, Y.-S., Pappas, N., 1999. Prediction of reinforcing responses to
10   psychostimulants in humans by brain dopamine D_2 receptor levels. Am. J. Psychiatry 156, 1440-1443.

Ward, W., 1997. Psychiatric morbidity in Australian veterans of the United Nations peacekeeping force in Somalia. Aust. N. Z. J. Psychiatry 31, 184-193.

Willner, P., 1997. The mesolimbic dopamine system as a target for rapid antidepressant
15   action. Int. Clin. Psychopharmacol. 12, Suppl 3, S7-S14.

Zatzick, D.F., Marmar, C.R., Weiss, D.S., Browner, W.S., Metzler, T.J., Golding, J.M., Stewart, A., Schlanger, W.E., Wells, K.B. 1997. Posttraumatic stress disorder and quality of life outcomes in a nationally representative sample of male Vietnam veterans. Am. J. Psychiatry, 154, 1690-1695.

20   Zygmont, M., Prigerson, H.G., Houck, P.R., Miller, M.D., Schear, M.K., Jacobs, S., Reynolds, C.F., 1998. A post-hoc comparison of paroxetine and nortriptyline for symptoms of traumatic grief. J. Clin. Psychiatry 59, 241-245.
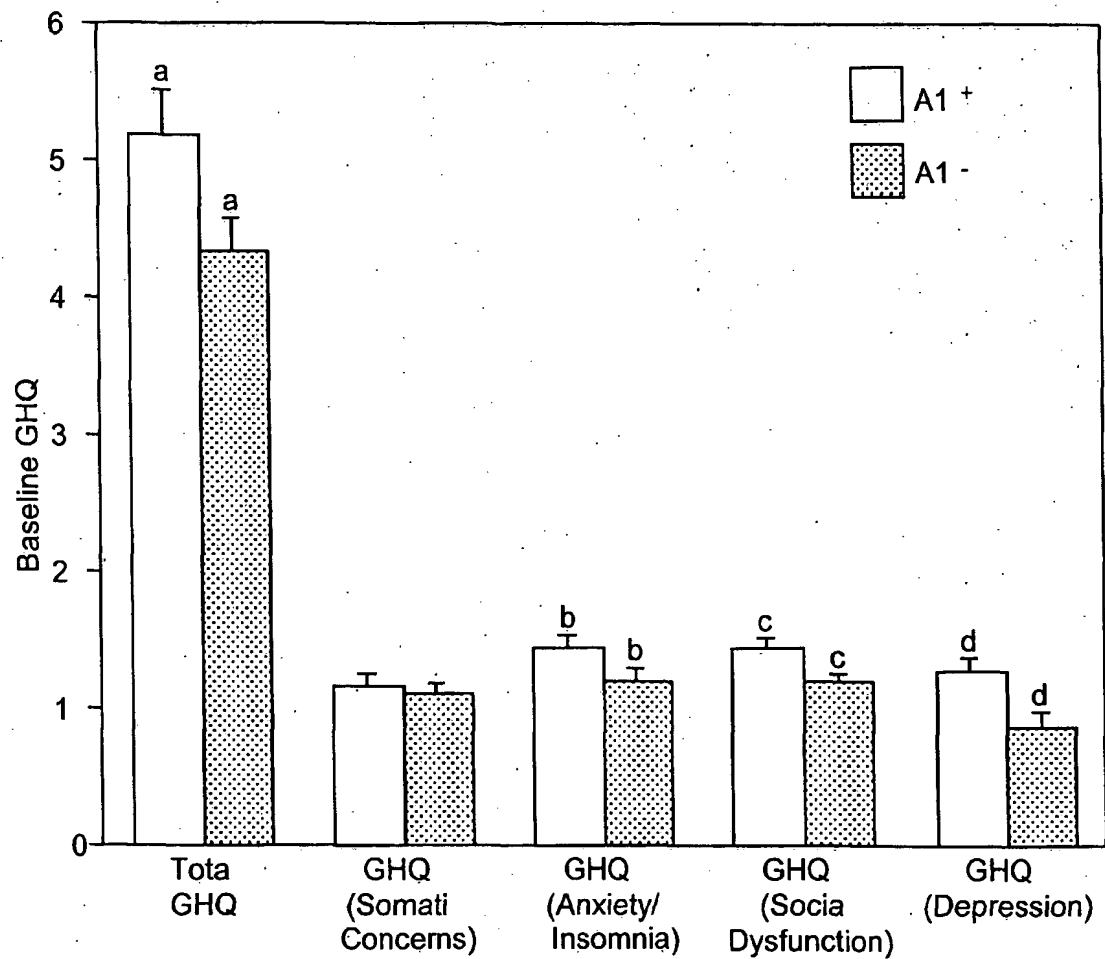
Figure 1 legend

Baseline and paroxetine treatment GHQ total scores of 45 PTSD patients and in 22 A1+ and 23 A1- DRD2 allelic patients.

a vs b, $t = 2.52$, $P = 0.015$

5   c vs d, $t = 2.82$, $P = 0.010$

e vs f, $t = 0.99$, $P = 0.334$

Example 4: The A1 allele of the D2 receptor is associated with comorbid depression and anxiety, in untreated veterans with combat-induced post traumatic stress disorder.

A total of 57 untreated Caucasian Vietnam veterans with combat-induced post traumatic stress disorder (PTSD) were administered the General Health Questionnaire-28. Cluster
5   analysis of these data identified two primary clusters. The first cluster, a high psychopathology cluster featured high comorbid levels of somatic concerns, anxiety, depression and social dysfunction. The low psychopathology cluster showed the reverse pattern. DRD2 A1+ (A1/A1, A1/A2 genotypes) veterans were significantly more likely to be found in the high psychopathology cluster ($\chi 2= 9.011$, $p<0.003$). There were no
10   significant differences between clusters in alcohol consumption. The results indicate that DRD2 variants are associated with comorbid psychopathology in PTSD. Implications of this finding regarding both the underlying pathophysiology and treatment of mixed anxiety depressive disorder (cothymia) are discussed.

Introduction

15   Combat-related posttraumatic stress disorder (PTSD) is a highly debilitating condition with a chronic course. The quality of life of PTSD patients is frequently compromised by comorbid conditions such as social anxiety disorder, panic disorder, generalized anxiety disorder, dysthymia and major depressive disorder (Zatzick et al., 1997; O'Toole et al., 1998). PTSD does not develop in all persons subjected to traumatic
20   stress suggesting considerable individual differences in susceptibility to this disorder (Stein et al 2002) and a genetic influence on symptoms remains even after accounting for combat exposure. Genetic factors contribute 13-30% of the variance in liability for re-experiencing symptoms, 30-34% of avoidant symptoms and 28-32% of hyperarousal symptoms (True, et al 1993). Risk of developing PTSD following combat trauma in
25   Vietnam was increased by a family history of paternal depression. In addition, pre-existing conduct disorder, panic disorder or generalised anxiety disorder and major depression were PTSD risk factors (Koenen et al., 2002). Individuals with PTSD report family psychiatric histories that closely resemble those found in sufferers of coexisting anxiety and depression. However, patients diagnosed with anxiety disorders alone and
30   control participants without psychiatric disorder report dissimilar family histories (Reich et al 1996). Importantly family history of psychiatric illness is found more frequently in combat veterans with comorbid depression and PTSD compared with those with PTSD alone. Stressor severity and number of traumatic events were not associated with comorbid depression (Kozaric-Kovacic, Hercigonja & Grubisic-Ilic, 2001).

35

A large twin study of 4072 male-male Vietnam veteran twins revealed that alcohol use and PTSD symptoms are associated and share a common genetic aetiological vulnerability (McLeod et al 2001). Combat veterans with PTSD carrying the A1 allele of the DRD2 (A1/A1 or A1/A2; A1+) consume twice as much alcohol at twice the rate
40   of those without this allele (A2/A2 or A1-) (Young et al 2002). Previous studies have found an inconsistent association between DRD2 variants and PTSD but have not examined the confound of possible substance misuse in the populations studied (Comings et al, 1996; Gelernter et al, 1999). As liability for alcohol problems and PTSD

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symptoms share a common genetic vulnerability and A1 + veterans have increased alcohol consumption we investigated whether differences in PTSD symptomatology was associated with A1+ status. Additionally, as PTSD subjects have family histories which are similar to those found in mixed anxiety depression we investigated whether
5 co morbid anxiety, depression, social dysfunction and somatic concerns was associated with A1+ individuals with PTSD.

10 Method
Fifty-five unrelated male Caucasian patients diagnosed with PTSD who were presenting for treatment were recruited for study. All subjects were Vietnam combat veterans who had served in the Australian armed forces. None were being treated with psychotropic medication. Patients were excluded from the study if they had a diagnosis of psychosis,
15 bipolar disorder, obsessive compulsive disorder, or organic brain syndrome including dementia. All subjects had sufficient comprehension of English and could understand the administered questionnaires.

Patients were assessed for PTSD by a consultant psychiatrist (BL) or a senior
20 psychiatric registrar (BK) using Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria. PTSD diagnosis was confirmed for all subjects. Furthermore, every patient exceeded the clinical cut-off score of 94 on the Mississippi Scale for combat-related posttraumatic stress disorder (Zatzick et al., 1997). The validity and reliability of the Mississippi scale are well established in veterans (Keane et al., 1988). Patients
25 then underwent clinical history taking by a psychiatrist or by a clinical nurse. Demographic data and ethnic background were also obtained.
The General Health Questionnaire–28 (GHQ) was administered. The GHQ-28 measures four psychopathological factors particularly relevant to PTSD and comorbid psychiatric conditions: somatic concerns (GHQ1), anxiety/insomnia (GHQ2), social dysfunction
30 (GHQ3) and depression (GHQ4). The GHQ has been widely validated internationally as a means of detecting psychiatric caseness and is sensitive to change (Ormel et al., 1989). The GHQ has utility as a follow-up measure of veteran mental health following exposure to combat (Deahl et al., 1994) and is sensitive to changes in combat-related PTSD symptoms (Ward, 1997) and has been used to assess symptom severity (Fuesner
35 et al 2001, Lawford et al, in press).
All participants provided written informed consent and were able to terminate the study without prejudice. Institutional ethics approval was obtained from the Greenslopes Private Hospital.

<p style="text-align:center">Genotyping</p>

40 A 10ml blood sample was drawn from each patient. Genomic DNA was extracted employing standard techniques and used as a template for determination of TaqI A DRD2 alleles by the polymerase chain reaction (Grandy et al., 1993). The amplification of DNA was carried out using a Perkin Elmer GeneAmp 9600 thermocycler. Approximately 500 ng of amplified DNA was digested with five units of TaqI

restriction enzyme (New England Biolabs) at 65°C overnight. The resulting products were separated by electrophoresis in a 2.5% agarose gel containing ethidium bromide and visualized under ultraviolet light. Three genotypes are obtained: the A1A2 genotype is revealed by three fragments: 310 bp, 180 bp and 130 bp. The A2A2

5   genotype by two fragments: 180 bp and 130 bp. The A1A1 genotype is shown by the uncleaved 310 bp fragment. A1+ allelic subjects are those that either have the A1A1 or A1A2 genotype; A1- allelic subjects have the A2A2 genotype only.

Information coded from the interviews, GHQ-28, Mississippi Scale and genotyping results were entered into a computer database. Nominal data were analysed by Yates

10   corrected X^2 test and continuous data were analysed by analysis of variance. A joining tree cluster analysis was employed to identify various symptom cluster groups (Everitt et al 2001). P values ≤ 0.05 were considered statistically significant. .

Results

The mean age of the 57 patients who entered treatment was 51.2 years. They had the

15   following genotypes: A1A2 (A1+ allele), $n = 22$; and A2A2 (A1- allele), $n = 35$. This genotype distribution did not deviate from the Hardy-Weinberg equilibrium ($\chi^2 = 2.86$, $P = 0.091$). There was no significant difference in the ages of A1+ (50.8 $\pm$ 0.7 years) and A1- (51.5 $\pm$ 1.0 years) allelic subjects ($F(1,55) = 0.23, P = 0.64$).

The GHQ data were analysed using joining tree cluster analysis in order to determine

20   subgroups of patients that differed according to symptom profile. These analyses were conducted blind to allelic status. The analysis showed four clusters and cluster scores for each of these are presented in Table 1. Cluster 1 (n=32) was characterised by low scores on all four GHQ factors, Cluster 2 (n=3) was characterised by low scores on somatic symptoms and social dysfunction, moderate scores on anxiety but significant

25   depressive symptomatology. Cluster 3 (n=16) was characterised by raised scores on all GHQ factors and Cluster 4 (n=6) showed significant somatic and anxiety symptoms with low scores on social dysfunction and an absence of depressive symptoms. Only two clusters, Cluster 1, the "low psychopathology" cluster and Cluster 3, the "high psychopathology" cluster had sufficient subject numbers for further statistical analysis.

30   Figure 1 presents mean Mississippi scores of Cluster1 and Cluster 3. Analysis of variance revealed Mississippi scores to be significantly higher in Cluster 3 when compared to Cluster 1 (Cluster 1, mean MISSI =121.3, s.d.=17.9, Cluster 3, mean MISSI =143.3, s.d.=13.5, F (1, 45) =18.75, p<0.0001) (see Figure 1) However there was no significant difference in alcohol consumption in grams per day between Cluster

35   1 (mean= 73.98 grams per day, s.d.= 124.89) and Cluster 3 (mean=131.75 grams per day, s.d = 140.56) (F(1,45) = 2.07, p=0.16). In the "low psychopathology" Cluster 1, 8 patients were A1+ and 24 patients were A1-. The "high psychopathology" Cluster 3 contained 12 patients who with A1+ status and 4 patients who with A1- status. Yate's corrected Chi-square statistic revealed that Cluster 3 had a significantly higher

40   prevalence of A1+ allelic participants than Cluster 1($\chi2$=9.011, p<0.003)

Table (1)

Cluster	GHQ1 (somatic	GHQ2 (anxiety	GHQ3 (social	GHQ4 (depression0

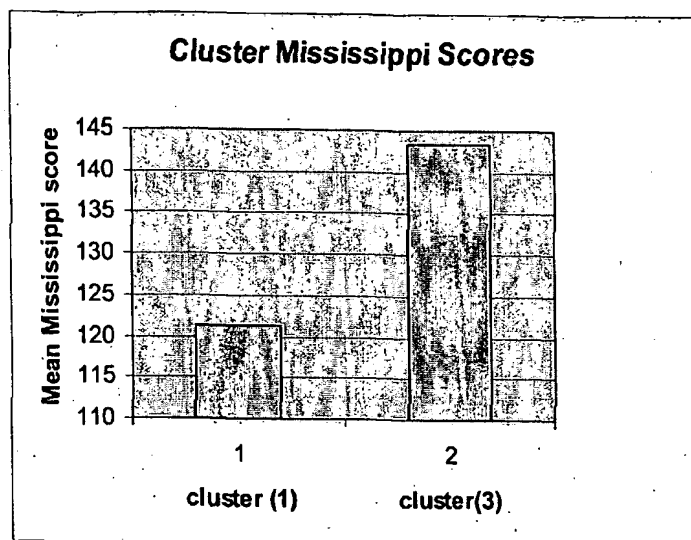|	symptoms)	symptoms)	dysfunction)	
1 (n=32)	-0.581	-0.767	-0.516	-0.525
2 (n=3)	-0.129	0.288	-0.788	1.331
3 (n=16)	0.803	1.153	1.131	1.115
4 (n=6)	1.023	0.87	0.120	-0.846

Figure 1 Mean Mississippi scores of Cluster1 and Cluster3
(Cluster1 vs. Cluster 3, $F(1,45) = 18.75$, $p<0.0001$)

5 DISCUSSION

The major finding of this paper was the strong association of A1+ status with comorbid somatic symptoms, anxiety, social dysfunction, and depression which was independent of alcohol effects. Furthermore, the identification of two main symptom clusters confirms a previous study (Hallman et al 2003)of 1161 Gulf War veterans where cluster
10   analysis again identified two primary groups. The first group reported good health and few moderate or severe symptoms. The second group was characterised by fair or poor health and increased frequency of moderate / severe symptoms.

Epidemiological studies and clinical experience show that the comorbidity of anxiety and depressive disorders is frequent (Bjelland, & Dahl, 1999). These observations have
15   led to the proposed establishment of a new disorder of mixed anxiety and depression (MAD) (Barlow & Campbell, 2000). MAD or cothymia has a significantly worse outcome than either an anxiety or depressive diagnosis alone. Furthermore, longitudinal data reveal social functioning in addition to anxiety and depression, is significantly worse in patients with cothymia compared to those with either anxiety or depression
20   alone (Tyrer et al, 2001).

The association of A1+ individuals with comorbid depression /anxiety/social dysfunction/somatic concerns implicates the involvement of D2 receptors in the coexistence of these disorders. There is compelling evidence that A1+ individuals have a reduced density of brain D2 dopamine receptors (Noble et al, 1991, Thompson et al,
25   1997, Pohjalainen et al, 1998, Jonsson, et al, 1999). An early brain autopsy study

(Noble et al., 1991) found a significant reduction of approximately 30% in the number of D2 dopamine receptors (Bmax) in the caudate nucleus of A1+ compared to A1-allelic subjects. Moreover, a significant progressive decline in Bmax was found across A2/A2, A1/A2, A1/A1 genotypes in that order. There was no difference in D2

5   dopamine binding affinity (Kd) between A1+ and A1- allelic subjects. Thompson et al., 1997 also reported 30%-40% reduction in D2 dopamine receptor density in the striatum of A1+ individuals compared to A1- individuals. An in-vivo study of healthy Finnish volunteers (Pohjalainen et al., 1998) showed significantly decreased D2 receptor density in the striatum of A1+ when compared with A1- allelic subjects. Again, there was no

10   difference in the Kd between the two groups. In Jonsson et al (1999), another PET study of healthy humans using (11C) raclopride, a significant association of the A1 allele with low D2 dopamine receptor density was found. While, Laruelle et al (1998) reported no association between reduced D2 binding and the A1 allele subjects an editorial (Hitzemann, 1998) suggested the study had insufficient power to detect a

15   significant difference between A1+ and A1- individuals.

The most likely explanation of the association of theA1 allele, a polymorphism in a non-coding region of the DRD2 gene, with reduced D2 receptor density, is that the sequence variation causing the Taq1 A polymorphism is in linkage disequilibrium with functional allelic variants that affect receptor expression (Noble, et al, 1998, Comings et al, 1991,

20   O'Hara et al 1993, Arinami et al, 1997). The Taq 1A variants are now known to be in linkage disequilibrium with C957T, a synonymous mutation in the human DRD2. Furthermore, C957T affects mRNA folding leading to both less stable mRNA and decreased translation. These effects dramatically diminish dopamine induced up-regulation of D2 receptors (Duan et al, 2003).

25   Both animal and human research implicate decreased D2 receptor functioning in the aetiology and treatment of depression and anxiety. Animal studies have reported that decreased caudate D2 –like receptor functioning is associated with learned helplessness which is a generally recognised model of depression (Kram et al 2002). Morphine can reverse a state of learned helplessness in rats and haloperidol, a D2 receptor antagonist,

30   blocks this action suggesting D2 receptor mediation of learned helplessness (Besson et al 1999). Social subordination in female cynomolgus monkeys is also associated with decreased D2 receptor function (Shively, 1998).

There are similar data regarding the role of the D2 receptor in the genesis of anxiety. In the rat amygdala both D1 and D2 receptors are involved in the mediation of the startle

35   response (Greba, Gifkins & Kokkinidis, 2001). The action of quinpirole, a D2 agonist, has confirmed that D2 dopamine receptors are involved in social and emotional activity in mice (Gendreau et al, 1998). Microinfusion of quinpirole suppresses the fear arousing properties of foot shock in rats (Gifkins et al, 2002). Dopamine D2 autoreceptor agonists inhibit ultrasonic vocalisation in rats reflecting an anxiolytic effect

40   in these animals (Bartoszyk, 1998).

Social phobia patients have low binding of [(123)I]IBZM to striatal D(2) receptors (Schneier et al, 2000). Furthermore there is strong linkage between the A1 allele for the D(2) receptor and the Tridimensional Personality Questionnaire (TPQ) harm avoidance scale. This scale largely reflects introversion (worry, pessimism and shyness) (Hill et al,

1999). Dopamine system hypoactivity has also been linked to trait detachment, social anxiety disorder and diminished reward and incentive function (Schneier et al, 2002). There is a significant relationship between low dopamine D2 receptor binding and NEO Personality Inventory-Revised non-clinical personality trait of depression (Kestler et al,

5   2000). Depressed patients exhibit a hypersensitive response to dextroamphetamine with severity of depression correlating highly with the rewarding effects of dextroamphetamine (Tremblay et al, 2002). Subjects administered intravenous methylphenidate experience reward that is inversely proportional to D2 receptor binding (Volkow et al., 1999, 2002).This suggests that severe depression is associated with

10   decreased brain D2 density.

Schizophrenics treated with typical antipsychotic drugs show worsening of depressive symptoms with increasing D2 receptor occupancy (Bressan et al, 2002). Similarly, a further PET study of patients with schizophrenia treated with the a-typical antipsychotics olanzapine or risperidone demonstrated that D2 receptor occupancy was

15   proportional to subjective experience of depression (de Haan et al, 2000). Furthermore, growth hormone response to apomorphine, a D2 agonist, is reduced in depressed patients who commit suicide reflecting reduced dopaminergic activity. (Pitchot et al, 2001).

The pharmacotherapeutic treatment of depression and anxiety states is similar. Selective

20   serotonin reuptake inhibitors (Berk, 2000, Rausch et al, 2001) and venlafaxine (Gorman and Papp, 2000) are effective in mixed anxiety and depression. . The therapeutic actions of these drugs and their adverse effects are due to neurotransmitter activating genes in target neurons (Stahl, 1999). Fluoxetine causes an increase in nucleus accumbens shell D2 dopamine receptor mRNA resulting in increased D2 dopamine

25   receptor postsynaptic binding in the nucleus accumbens (Ainsworth et al., 1998). Similarly, the SSRI citalopram increases D2 dopamine receptor mRNA in the striatum and nucleus accumbens (Dziedzicka-Wasylewska et al., 1997). This effect of citalopram on increased transcription of the D2 dopamine receptor gene is likely to be mediated by increased serotonin levels induced by SSRIs, as 5 hydroxytryptophan also

30   increases D2 dopamine receptor mRNA transcription (Kameda et al., 2000). Dopamine D2 receptor binding increases in SSRI treatment responders and decreases in non responders (Klimke et al, 1999). suggesting that induction of D2 dopamine receptor gene expression is likely to be an important mechanism by which SSRIs exert therapeutic effects.

35   Dopamine receptor ligands also have both anxiolytic and antidepressant effects (Bartoszyk, 1998, Theohar 1982)). A double-blind placebo controlled study (Lawford et al, 1995) examined the effects of a D2 dopamine receptor agonist, bromocriptine (BRO) and placebo (PLA) on treatment outcome in alcoholism. The results showed that in the four groups of alcoholics studied (BRO A1+, BRO A1-, PLA A1+, PLA A1-) the

40   greatest and most significant decreases in craving and anxiety were found in A1+ alcoholics treated with bromocriptine (BRO A1+). Bromocriptine has similar efficacy as imipramine and amitriptyline for the treatment of endogenous depression (Bouras, Bridges et al, 1982 & Waehrens & Gerlach, 1981, Theohar et al, 1982).

Considerable evidence from both animal and human studies emphasises the importance of low D2 receptor functioning in both anxiety and depression. It is not therefore surprising that the A1 allele for the D2 receptor that is genotypically expressed as reduced D2 density is associated with comorbid severe anxiety and depression in combat veterans with PTSD. Further investigation of this polymorphism in anxiety/depressive disorders may improve understanding of the pathophysiology of these states and eventually lead to targeted pharmacogenetic treatment interventions.

References

Ainsworth, K., Smith, S.E., Zetterstrom, T.S., Pei, Q., Franklin, M., Sharp, T., 1998. Effect of antidepressant drugs on dopamine D1 and D2 receptor expression and dopamine release in the nucleus accumbens of the rat. Psychopharmacology 140, 470-477.

Arinami T, Gao M, Hamaguchi H, Toru M,(1997) A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. Human Molecular Genetics 6:577.

Barlow, DH, Campbell, L.A, 2000. Mixed anxiety-depression and its implications for models of mood and anxiety disorders. Compr Psychiatry. 41(2 Suppl 1) 55-60.

Bartoszyk, GD, 1998. Anxiolytic effects of Dopamine Receptor Ligands: I. Involvement of dopamine autoreceptors. LifeSci 62:649-63

Berk, M, 2000. Selective serotonin reuptake inhibitors in mixed anxiety-depression. Int Clin Psychopharmacol. 15 Suppl 2: S41-5.

Besson A, Privat AM, Eschalier A, Fialip J 1998. Reversal of learned helplessness by morphine in rats: involvement of a dopamine mediation. Pharmacol Biochem Behav 60:519-25

Bjelland, I, Dahl, AA, 1999. Anxiety and Depression – a common mixture. Tidsskr Nor Laegeforen. 119: 3277-80.

Bouras, N, Bridges, PK. 1982. Bromocriptine in depression. Curr Med Res Opin 8:150-153.

Bressan, RA, Costa, DC, Jones HM, Ell PJ, Pilowsky, LS, 2002. Typical antipsychotic drugs-D2 receptor occupancy and depressive symptoms in schizophrenia. Schizophr Res. 56: 31-6.

Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, Knell E, Kocsis P, Baumgarten R, Kovacs BW, Levy DL, Smith M, Borison RL, Evans DD, Klein DN, MacMurray J, Tosk JF, Sverd J, Gysin R, Flanagan SD,. (1991) The dopamine D2 locus as a modifying gene in neuropsychiatric disorders. Journal of the American Medical Association. 266:1793-1800

Comings, DE, Muhleman, D & Gysin, R (1996). Dopamine D2 receptor (DRD2) gene and susceptibility to posttraumatic stress disorder: a study and replication. Biol Psychiatry, 40, 386-72.

Deahl, M.P., Gillham, A.B., Thomas, J., Searle, M.M., Srinivasin, M., 1994. Psychological sequelae following the Gulf War. Factors associated with subsequent morbidity and the effectiveness of psychological debriefing. Br. J. Psychiatry 165, 60-65.

de Haan, L, Lavalaye, J, Linszen, D, Dingemans, PM, Booij, J, 2000. Subjective experience and striatal dopamine D(2) receptor occupancy in patients with schizophrenia stabilized by olanzapine or risperidone. Am J Psychiatry 57:1019-20..

Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV (2003) Synonomous mutations in the human dopamine D2 (DRD2) affect mRNA stability and synthesis of the receptor. Human Molecular Genetics 12:205-216.

Dziedzicka-Wasylewska, M., Rogoz, R., Klimek, V., Maj, J., 1997. Repeated administration of antidepressant drugs affects the levels of mRNA coding for D1 and D2 dopamine receptors in the rat brain. J. Neural. Transm. 10, 515-524.

Everitt BS, Landau S, Leese M ;. 2001. Cluster Analysis. London; Edward Arnold

Fuesner, J, Ritchie, T, Lawford, B, Young, RM, Kann, B & Noble EP (2001). GABA-A beta 3 subunit gene and psychiatric morbidity in a post-traumatic disorder stress population. Psychiatry Research, 104, 109-17.

Gelernter, J, Southwick S, Goodson, S, Morgan A, Nagy L, Charney, DS (1999). No association between D2 dopamine receptor (DRD2) "A" system alleles, or DRD2 haplotypes, and posttraumatic stress disorder. Biol Psychiatry, 45, 620-5.

Gendreau, PL, Petitto, JM, Gariepy, JL , Lewis, MH (1998). D2 – like dopamine receptor mediation of social emotional reactivity in a mouse model of anxiety: strain and experience effects. Neuropsychopharmacology, 18, 210-21.

Gifkins, A, Greba Q, Kokkinidis, L (2002). Ventral tegmental area dopamine neurons mediate the shock sensitization of acoustic startle: a potential site of action for benzodiazepine anxiolytics. Behav Neurosci 116: 785-94

Gorman JM, Papp LA. 2000 Efficacy of venlafaxine in mixed depression-anxiety states. Depress Anxiety. 12 Suppl 1:77-80.

Greba Q, Gifkins, A, Kokkinidas, L (2001). Inhibition of amygdaloid dopamine D2 receptors impairs emotional learning measured with fear-potentiated startle. Brain Research, 899, 218-26.

Grandy, D.K., Zhang, Y., Civelli, O., 1993. PCR detection of the Taq A RFLP at the DRD2 locus. Hum. Mol. Genet. 2, 2197.

Hallman WK, Kipen HM, Diefenbach M, Boyd K, Kang H, Leventhal H, Wartenberg D. 2003 Symptom Patterns Among Gulf War Registry Veterans. Am. J. Public Health;93:624-630

Hill, SY, Zezza, N, Wipprecht, G, Locke, J, Neiswanger, K. 1999. Personality traits and dopamine receptors (D2 and D4): linkage studies in families of alcoholics. Am J Med Genet 88:634-41.

Hitzemann, R.J., 1998. The regulation of D_2 dopamine receptor expression. Mol. Psychiatry 3, 198-203.

Jönsson, E.G., Nöthen, M.M., Grünhage, F., Farde, L., Nakashima, Y., Propping, P., Sedvall, G.C., 1999. Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. Mol. Psychiatry 4, 290-296.

Kameda, K., Kusumi, I., Suzuki, K., Miura, J., Sasaki, Y., Koyama, T., 2000. Effects of citalopram on dopamine D2 receptor expression in the rat brain striatum. J. Mol. Neurosci. 14, 77-86.

Keane, T.M., Caddell, J.M., Taylor, K.L., 1988. Mississippi Scale for combat-related posttraumatic stress disorder: three studies in reliability and validity. J. Consult. Clin. Psychol. 56, 85-90.

Kestler, LP, Malhotra, AK, Finch C, Adler, C, Breier, A. 2000. The relation between
5   dopamine D2 receptor density and personality: preliminary evidence from the NEO personality inventory-revised. Neuropsychiatry Neuropsychol Behav Neurol 13:48-52.

Klimke, A., Larisch, R., Janz, A., Vosberg, H., Muller-Gartner, H.W., Gaebel, W., 1999. Dopamine D2 receptor binding before and after treatment of major depression measured by [^{123}I] IBZM SPECT. Psychiatry Res. Neuroimaging. 90, 91-101.

10   Koenen KC, Harley R, Lyons MJ, Wolfe J, Simpson JC, Goldberg J, Eisen SA, Tsuang M. 2002 A twin registry study of familial and individual risk factors for trauma exposure and posttraumatic stress disorder. J Nerv Ment Dis ; 190:209-218

Kozaric-Kovacic D, Hercigonja DK & Grubisic-Ilic M (2001). Posttraumatic stress disorder and depression in soldiers with combat experiences. Croat Med J, 42, 165-70.

15   Kram ML, Kramer GL, Ronan PJ, Steciuk M, Petty F. (2002) Dopamine receptors and learned helplessness in the rat: an autoradeiographic study. Prog Neuropsychopharmacol Biol Psychiatry ;26:639-45

Laruelle, M., Gelernter, J., Innis, R.B., 1998. D$_2$ receptors binding potential is not affected by TaqI polymorphism at the D$_2$ receptor gene. Mol. Psychiatry 3, 261-265.

20   Lawford et al Paroxetine paper (see Example 3)

Lawford, B.R., Young, R.McD., Rowell, J.A., Qualichefski, J., Fletcher, B.H., Syndulko, K., Ritchie, T., Noble, E.P., 1995. Bromocriptine in the treatment of alcoholics with the D2 dopamine receptor A1 allele. Nature Med. 1, 337-341.

McLeod, D.S, Koenen, K.C., Meyer, J.C., Lyons, M.J., Eisen, S., True, W. & Goldberg,
25   J (2001). Genetic and environmental influences on the relationship among combat exposure and posttramatic stress disorder symptoms and alcohol use. J Traum. Stress. 14, 259-75.

Noble, E.P., Blum, K., Ritchie, T., Montgomery, A., Sheridan, P.J., 1991. Allelic association of the D2 dopamine receptor gene with receptor binding characteristics in
30   alcoholism. Arch. Gen. Psychiatry, 48, 648-654.

Noble, EP, Zhang, X, Ritchie, T, Lawford B, Grosser, SC, Young, RMcD & Sparkes RS (1998). D2 dopamine receptor and GABA-A receptor β3 subunit genes and alcoholism. Psychiatry Research, 81, 133-147.

O'Hara, BF, Smith SS, Byrd G, Persico, AM, Suarez, BK, Catting, GR, Uhl, G (1993).
35   Dopamine D2 receptor RFLPS haplotypes and their association with substance use in black and Caucasian research volunteers. Hum Hered 43: 209-218.

Ormel, J., Koeter, M.W., van der Brink, W., Giel, R., 1989. Concurrent validity of GHQ-28 and PSE as measures of change. Psychol. Med., 19, 1007-1013.

Otani S, Auclair N, Desce JM, Roisin MP, Crepel F.1999. Dopamine receptors and
40   groups 1 and 11 mGluRs cooperate for long term depression and induction in rat prefrontal cortex through converging postsynaptic activation of MAP kinases. J Neurosci, 19 , 9788-802.

O'Toole, B.I., Marshall, R.P., Schureck, R.J., Dobson, M., 1998. Posttraumatic stress disorder and comorbidity in Australian Vietnam veterans: risk factors, chronicity and
45   combat. Aust. N. Z. J. Psychiatry, 32, 32-42.

Pitchot, W, Reggers, J, Pinto, E, Hansenne, M, Fuchs, S, Pirard, S, Ansseau, M. 2001. Reduced dopaminergic activity in depressed suicides. Psychoneuroendocrinology. 26:331-5.

Pohjalainen T, Rinne JO, Nagren K, Lehikoinen P, Anttila K, Syvalahti EK, Hietala J.(1998) The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. Mol Psychiatry , 3:256-60 .

Rausch, JL, Hobby, HM, Shendarkar, N, Johnson, ME, Li, J. 2001. Fluvoxamine treatment of mixed anxiety and depression: evidence for serotonergically medicate anxiolysis. J Clin Psychopharmocol. 21: 139-42.

Reich J, Lyons M & Cai B (1996). Familial vulnerability factors to post-traumatic stress disorder in male military veterans. Acta Psychiatrica Scand, 93, 105-12.

Schneier, FR, Liebowitz, MR, Abi-Dargham, A, Zea-Ponce, Y, Lin, SH, Laruelle, M 2000. Low dopamine D(2) receptor binding potential in social phobia. Am J Psychiatry 58:237-28

Schneier, FR, Blanco, C, Antia, SX, Liebowitz, MR. 2002. The social anxiety spectrum. Psychiatr Clin North Am 25: 757-74

Shively CA (1998). Social subordination stress, behaviour, and central monoaminergic function in female cynomolgus monkeys. Biol Psychiatry, 44, 882-91.

Stahl, S.M., 1999. Molecular neurobiology for practicing psychiatrists, part 4: transferring the message of chemical neurotransmission from presynaptic neurotransmitter to postsynaptic gene expression. J. Clin. Psychiatry 60, 813-814.

Stein MB, Jang KL, Taylor S, Vernon PA, Livesley WJ. 2002 Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms : a twin study. Am J Psychiatry 159:1675-81

Theohar, C, Fischer-Cornelssen, K, Brosch, H, Fischer, EK, Petroic, D. 1982. A comparative, multicenter trial between bromocriptine and amitriptyline in the treatment of endogenous depression. Arzneimittelforschung 32:783-787.

Thompson J, Thomas N, Singleton A, Piggott M, Lloyd S, Perry EK, Morris CM, Perry RH, Ferrier IN, Court JA.(1997) D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine receptor binding in the human striatum associated N, Singleton A, Piggott M, Lloyd S, Perry EK, Morris CM, with the A1 allele., Pharmacogenetics ,7:479-84

Tremblay, LK, Naranjo, CA, Cardenas, L, Herrmann, N, Busto, UE. 2002. Probing brain reward system function in major depressive disorder: altered response to dextroamphetamine. Arch Gen Psychiatry 2002. 59:409-16.

True WR, Rice J, Eisen SA, Heath AC, Goldberg J, Lyons MJ, Nowak J (1993) A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. Arch Gen Psychiatry 50;257-64

Tyrer, P, Seivewright, H, Simmonds, S, Johnson, T. 2001. Prospective studies of cothymia (mixed anxiety-depression): how do they inform clinical practice? Eur Arch Psychiatry Clin Neurosci. 251 Suppl 2: 1153-6.

Volkow, N.D., Wang, G.J., Fowler, J.S., Logan, J., Gatley, S.J., Gifford, A., Hitzemann, R., Ding, Y.-S., Pappas, N., 1999. Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D_2 receptor levels. Am. J. Psychiatry 156, 1440-1443.

Volkow, ND, Wang G-J, Fowler, JS, Thanos T, Logan. J, Gatley, SJ, Gifford, A, Ding, Y-S, Wong C & Pappas, N (2002). Brain DA D2 receptors predict reinforcing effects of stimulants in humans: replication study. Synapse, 46; 79-82

5   Waehrens, J, Gerlach, J. 1981. Bromocriptine and imipramine in endogenous depression: A double-blind controlled trial in outpatients. J Affect Disord 3:193-202.

Ward, W., 1997. Psychiatric morbidity in Australian veterans of the United Nations peacekeeping force in Somalia. Aust. N. Z. J. Psychiatry 31, 184-193.

Young, RM, Lawford, BR, Noble, EP, Kann, B, Wilkie, A., Ritchie, T, Arnold, L & Shadforth, S (2002). Harmful drinking in military veterans with post-traumatic stress

10   disorder: association with the D2 dopamine receptor A1 allele. Alcohol Alcohol, 37, 451-6.

Zatzick, D.F., Marmar, C.R., Weiss, D.S., Browner, W.S., Metzler, T.J., Golding, J.M., Stewart, A., Schlanger, W.E., Wells, K.B. 1997. Posttraumatic stress disorder and quality of life outcomes in a nationally representative sample of male Vietnam veterans.

15   Am. J. Psychiatry, 154, 1690-1695.

From the foregoing it will be appreciated that, although specific embodiments of the

20   invention have been described herein for purposes of illustration, various modifications

may be made without deviating from the spirit and scope of the invention. Accordingly,

the invention is not limited except as by the appended claims.

CLAIMS

What is claimed is:

1.   A method of identifying a candidate psychiatric patient for treatment with

medication that acts at a D2 dopamine receptor or influences D2 dopamine

5   receptor density, the method comprising:

        determining whether the patient's DRD2 genotype is Taq1A allele positive
    (A1+);

        wherein an A1+ genotype is indicative of a candidate for treatment with

highdose high DRD2 binding atypical antipsychotics and/or SSRIs that increase D2

10   dopamine receptor density; and

        wherein an A1- genotype is indicative of a candidate for treatment with lowdose

high or low D2 dopamine receptor binding atypical antipsychotics or alternative

antidepressant.

2.   The method of claim 1, wherein the psychiatric patient suffers from

15   schizophrenia.

3.   The method of claim 1, wherein the patient suffers from PTSD, depression,

social anxiety or mixed anxiety and depressive states.

4.   The method of claim 1 when the patient suffers from a movement disorder, such

as Parkinson's Disease.

20

ABSTRACT OF THE DISCLOSURE

The invention provides methods of identifying candidate psychiatricpatients, or

5   patientswith movement disorder, for treatment with medication that acts at a D2

dopamine receptor site or increases density of D2 dopamine receptors. The method

comprises determining a patient's DRD2 genotype. Patients having the Taq1A (A1)

allele (A1+ allelic status) are candidates for treatment with highdose, high binding

antipsychotics and/or SSRIs that influence D2 receptor density. Patients lacking the

10   Taq1A allele (A1- allelic status) are candidates for treatment with lowdose, low low

binding atypical antipsychotics, and are not likely to respond well tothese SSRIs.